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BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

F. C. BAWDEN D. J. FINNEY

J. W. EVANS H. MARTIN

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ERRATUM

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Lines 5 and 6, column 2, page 15 *should read*:

The mean for infected plants (averaging all rates and dates of infection) was $0{\cdot}53 \pm 0{\cdot}015\,\%$, compared with $0{\cdot}46 \pm 0{\cdot}026\,\%$ for healthy plants.

The quantity and distribution of spray collected by insects flying through insecticidal mists*

BY W. A. L. DAVID, *A.R.C. Unit of Insect Physiology, Entomological Field Station*, 34a *Storey's Way, Cambridge*

(With Plate 9 and 2 Text-figures)

When a housefly (*Musca domestica* L.) or the yellow-fever mosquito (*Aëdes aegypti* L.) flies through a finely dispersed insecticidal mist a large proportion of the dose accumulated is found on the wings. If Sudan III is added to the insecticide the dye either penetrates into the wings directly and later appears in the malpighian tubules or it may be removed during the cleaning processes and absorbed through the legs.

A colorimetric method of estimating the quantity of spray accumulated by flying insects is described, and it has been employed to investigate the effect which changes in the properties of the mist dispersions and the behaviour of the insects have on the quantity of spray accumulated. Briefly it may be said that within limits the dose accumulated increases with the activity of the insect and the size of the mist particle. The method has also been used to measure the surface median lethal doses of pyrethrins, 2:2-bis (*para*chlorophenyl)-1:1:1-trichlorethane (D.D.T.) and 1:2:3:4:5:6-hexachloro*cyclo*hexane (666) to *Musca* and *Aëdes*.

INTRODUCTION

When an insect is exposed to an insecticidal spray mist, and it collides with the droplets, the energy of the impacts involved may be largely derived from the flight movements of the insect or from the movements of the droplets. Neither the insect nor the droplets move in a simple constant manner, so that the relationship between the mist and the insect is complex and constantly changing. For example, the insects may vary the frequency and amplitude of their wing beats and so their rate of flight, while the movements of the spray droplets will change as they evaporate and come under the influence of convection currents.

The present paper is mainly concerned with the situation in which the flight movements of the insects are largely responsible for the collisions between the spray droplets and the insects. Such a situation occurs when insects fly through a mist of relatively stationary droplets of particle size below about 10 μ diameter. It has been shown elsewhere that under these circumstances the quantity of spray accumulated is dependent upon the particle size of the mist and also on the intensity of flight activity (David & Bracey, 1944, 1946). However, even with a knowledge of the behaviour of the insects, the exposure conditions, and the properties of the spray mist it is impossible to make more than the most general statements concerning the quantity of spray which will be collected. The need for a method of measuring the quantity of spray which flying insects accumulate from a mist dispersion is therefore obvious. Before describing the method which has been developed and its applications, certain preliminary observations on the distribution of the spray droplets collected by insects exposed to dyed mists are presented.

EQUIPMENT AND METHODS

A full description of the spray chamber and accessory equipment employed in the course of this work has been given elsewhere (David, 1946*a*). It consisted of an inner metal chamber measuring 42 × 48 × 48 in. high (volume 54·5 cu.ft. = 1543 l.) surrounded by a constant-temperature cabinet. The chamber was adjusted to run at 28° C., and before each test the relative humidity was brought to 70% by blowing in steam. The spray was applied near the top of the chamber from an Aerograph M.P. gun fitted with a no. 1 nozzle which was operated at 12·5 lb./sq.in. air pressure. After the spray had been injected the insects were introduced into the spray chamber enclosed in cages through one of four glass windows in the front of the cabinet. The cages rested on a shelf within the chamber, and the insects could be observed through the gauze ends of the exposure cages. The properties and behaviour of spray mists in this chamber have also been considered elsewhere (David, 1946*b*).

The test insects

The test insects employed were either *Aëdes aegypti* L. reared according to the method previously described (David *et al.* 1944), or *Musca domestica* L.

* This paper represents a portion of a thesis approve d for the degree of Doctor of Philosophy in the University of London.

bred by a modification of the Peet-Grady technique (*Soap Blue Book*, 1944), in which alfalfa meal was replaced by grass meal and dried blood. (I am indebted to Dr H. Bovingdon for the receipt of this modified formula.)

Assessment and treatment of the results

Where the results of experiments take the form of percentage kills these were determined between 18 and 24 hr. after the test exposures, separate assessments being made for males and females. Any insects which showed the slightest signs of movement were counted as alive. The percentage kills were sometimes converted to angles on which an analysis of variance was carried out (Bliss, 1938). In other cases the probit/log-dosage conversion was used to draw provisional regression lines (Bliss, 1935) from which values for median lethal dosages were taken.

Observations on the distribution of spray droplets collected by flying insects

Apparently little attention has been paid to the distribution of the dose of spray on insects following an exposure to a spray mist, although Murray (1940) measured the total dose accumulated by individual flies in the Peet-Grady test.

Insects exposed to oil sprays

When insects are allowed to fly through a mist of odourless kerosene dyed with Sudan III and preferably containing up to about 5% lubricating oil to retard evaporation applied at a dosage equivalent to 55 c.c./100 cu.ft. and in which all droplets are below 20 μ in diameter, it is immediately apparent that a very heavy dose is accumulated on the wings. It can be seen (Pl. 9, fig. 1 *b*) that the droplets do not spread over the wing surface, but form irregular flattened patches between about 2 and 20 μ in diameter, and a closer inspection suggests that they are, in part at any rate, held by the microtrichia. The droplets are distributed all over the wings on both surfaces but tend to be more numerous near the tips and posterior margins. There is always a heavier deposit on the wing tip if this is slightly damaged. In the housefly the squamae are also heavily coated, and in both the mosquito and the housefly the halteres, which vibrate in flight, also become dyed. No droplets are visible on any other parts of the body of either houseflies or mosquitoes, and separate extraction of the wings and bodies, followed by colorimetric comparison, shows clearly that very much more spray is accumulated on the wings than on the rest of the body (see quantitative estimation later). The fact that no droplets can be observed on the body does not necessarily mean that none has impacted; it may merely mean that the droplets have spread over the surface. The different behaviour of small oil droplets on the wings from that on the rest of the body surface may be confirmed by touching these parts with the smallest possible oil droplets carried on a fine dissecting needle. On the abdomen and legs they spread quickly, but on the wings they are held in thick patches between the microtrichia.

Insects are reluctant to fly with a heavy dose of oil-spray droplets on their wings, and as soon as they land after flying through an insecticidal mist they begin to clean themselves. The forelegs are used to clean the head and antennae while the hindlegs clean the wings. This cleaning process is carried out by both *Musca* and *Aëdes* and results in the removal of much of the accumulated spray. The process will be followed quantitatively later, but it can be readily seen that after exposure to a non-lethal spray, many of the droplets are removed within the course of a few hours. Even within a few minutes after exposure to a spray streaks of dyed oil may be observed on the walls of the exposure cage which must have been deposited from the legs or by contact with the wing tips. It is therefore clear that unless the insect is paralysed quickly by the spray it will transfer much of the dose accumulated to its surroundings.

When working with sprays dyed with Sudan III it was observed that the treated flies deposited dyed faeces on the floors of the recovery cages. At first it was thought that the dye represented material removed from the forelegs by the proboscis, and careful observations on a large number of flies showed that dyed spray did occasionally enter the proboscis, but the majority of the dye reached the gut by penetrating through the body wall to the haemocoele from where it was collected by the malpighian tubules as the following experiments show.

A batch of houseflies about 5 days old was divided into several groups which were treated as follows. All were lightly chloroformed and transferred to a chilled dish to retard recovery.

Group (*a*): Control flies which were allowed to recover but were not exposed to spray.

Group (*b*): Normal flies allowed to recover and then exposed to spray.

Group (*c*): Flies with anus and proboscis blocked with wax allowed to recover and then exposed to spray.

The spraying was carried out in the usual way with 0·7 c.c. of spray solution consisting of 0·3% D.D.T. plus 1% Sudan III in odourless kerosene. During the test the cage in which the various groups were exposed together was shaken to increase the flight activity. In two experiments normal flies and insects with their orifices blocked showed identical kills. When examined 24 hr. later group (*a*) showed no suggestion of red colouring matter in gut and no

red-coloured excrement in the recovery cages, group (*b*) showed a residue of red dye in the gut and very numerous dyed excrement marks in the recovery cage, group (*c*) showed a large quantity of dye in the rectum and malpighian tubules but no deposits of faeces in the recovery cages. It is therefore obvious that Sudan III which is voided in the excrement represents material which has penetrated through to the integument.

A further experiment was designed to investigate the rate at which Sudan III penetrated into the housefly. The previous experiment was repeated with normal flies which were examined either immediately after the test exposure, 60 min. later or 24 hr. later. The distribution of the dye after these intervals is shown in Table 1. It will be noted that within 1 hr. the dye has penetrated from the integument, passed through the body cavity, been accumulated in the malpighian tubules, and passed to the rectum.

TABLE 1. *Distribution of dye on houseflies exposed to a spray of Sudan III in odourless kerosene and examined after the intervals indicated*

The distribution of dye is indicated as follows: ++ strong, + medium, ± weak, − absent.

	Time at which examination was made		
Region examined	0·25 hr.	1 hr.	24 hr.
Wings	++	++	+
Pulvilli	++	++	±
Proboscis	±	−	−
Malpighian tubules	−	++	±
Rectum	−	++	+
Floor of recovery cage	−	±	++

Insects exposed to water sprays

Since it had initially seemed probable that oil droplets picked up on an insect would spread and not be visible a test was made in which the insects were exposed to a spray consisting of a 5% solution of gum arabic in water dyed with eosin. By applying this spray directly to the body and wings of chloroformed houseflies it could be seen that it did not spread in either case but occurred as perfectly spherical droplets (Pl. 9, fig. 1*a*). As would be expected when insects fly through this mist numerous droplets accumulate on the wings, but they are also visible on other parts of the body if the insects are chloroformed before they have an opportunity to clean themselves. This is especially true of *Musca*. Thus droplets can be found on the antennae (Pl. 9, fig. 2), eyes, mouthparts, large body bristles, the halteres, and on the fine hairs guarding the entrance to the anterior spiracles. There are not very many to be found on any of these places. When the lower surface of the abdomen of *Musca* is examined after exposure to the mist it is seen to have collected a very large number of droplets. The anterior and posterior pairs of legs (especially the latter) also show heavy collections of droplets which have presumably been combed from the head and wings respectively during exposure and before the insects could be chloroformed. One of the tarsal joints of the hindleg of *Musca*, on which there are a large number of droplets partly held by the comb of bristles, is shown in Pl. 9, fig. 3.

The situation in *Aëdes* is rather different. Droplets are again collected on the wings and a few may be found on the antennae and the halteres, but the abdomen appears to collect very few, except around the tip where appreciable numbers may be found.

QUANTITATIVE OBSERVATIONS ON THE SPRAY COLLECTED BY FLYING INSECTS

Method

The method employed in estimating the quantity of spray accumulated by insects flying through an insecticidal mist consisted of dyeing the spray with a known quantity of Sudan III and subsequently extracting the treated insects in odourless kerosene. Sudan III can be completely dissolved in odourless kerosene only with considerable difficulty, and in the preparation of the sprays it was dissolved in benzene, and this solution was added to the insecticide in odourless kerosene so that the spray contained 50% of each solvent. The solutions obtained by extracting the insects were compared with a set of standards prepared by diluting the spray solution with odourless kerosene. From a knowledge of the amount of dye recovered from the insects it was possible to calculate the quantity of insecticide present on the assumption that with relatively non-volatile materials such as pyrethrins, 2:2-bis (*para*chlorophenyl)-1:1:1-trichlorethane (D.D.T.) and hexachloro*cyclo*hexane (666), the ratio of dye to insecticide would not have undergone any appreciable change.

The standards, each 1 c.c. in volume, were contained in small hard glass tubes and covered the range 0–1·1 μg. of Sudan III per c.c. usually at intervals of 0·2 μg., starting from 0·1 μg./c.c. The number of insects employed and the volumes of kerosene used for the extractions were adjusted to bring the experimental samples within the range indicated.

The properties of the spray mist in relation to the dose accumulated by flying insects

(*a*) *The influence of the age of the spray mist on its insecticidal efficiency*

As the interval after formation of a spray mist increases, the number and size of the droplets

present decrease due to precipitation and evaporation and there is a corresponding decline in insecticidal efficiency (David, 1946*b*).

By means of the colorimetric method just described it is possible to show that the decrease in insecticidal efficiency is due to the accumulation of a smaller dose of insecticide by the flying insects as would of course be expected. Sprays were prepared as already described containing 1·0 % w/v of Sudan III and either 0·1 % w/v of pyrethrins, 0·3 % w/v of D.D.T. or 0·3 % w/v of 666. The two latter sprays contained 0·01 % pyrethrins to stimulate the insects into activity. The sprays were injected into the chamber and the insects, *Aëdes aegypti*, were given a 10 min. exposure commencing 0·5, 4·0 or 10 min. after spraying. Half of the exposed batch were kept for extraction, and the remainder were transferred to recovery cages. The percentage kill of males and females was determined after 24 hr. and averaged. The quantity of spray collected by each sex was also measured separately and averaged—in Table 2 it is expressed in mg. of pure insecticide per kg. of mosquitoes.

TABLE 2. *The decrease in insecticidal efficiency of a spray mist with age. The figures show the time after spraying at which the exposure of the insects was commenced, the dose of insecticide accumulated and the percentage kill of* Aëdes aegypti *recorded. Further details are given in the text*

Age of mist (min.)	Pyrethrins		D.D.T.		666	
	Dose (mg./kg.)	Kill (%)	Dose (mg./kg.)	Kill (%)	Dose (mg./kg.)	Kill (%)
0·5	2·5	87	16·0	89	4·5	87
4·0	1·5	66	7·0	52	3·5	68
10·0	0·75	46	5·0	41	1·5	5

(*b*) *The influence of the particle size of the spray mist on the dose accumulated*

It has already been reported that the addition of about 5 % v/v of non-volatile ingredient to insecticidal sprays leads to the production of mists in which particles of a larger size persist than occur in the absence of the non-volatile material, due to the failure of the latter to evaporate (David & Bracey, 1944). Where this difference in particle size becomes established it is associated, as might be expected, with an increase in the insecticidal efficiency of the mist having the larger particles since these impact more readily on the flying insect. The colorimetric technique provides a means of demonstrating conclusively that more insecticide is accumulated from coarser mists.

Two spray solutions were prepared containing the same quantity of pyrethrins (0·045 % w/v) and Sudan III (1·0 % w/v), while one contained in addition 5·0 % v/v of lubricating oil (high-vacuum pump oil). These sprays were injected into the spray chamber in equal quantities, and batches of insects were exposed to either under identical conditions. The relative quantities of insecticide accumulated from each spray and the resultant kills in 24 hr. are shown in Table 3, while photographs of the wings of the exposed insects are presented in Pl. 9, figs. 4*a*, *b*. The apparent disagreement between the dosage and kill given in Table 3, and the median lethal dose of pyrethrins to *Aëdes* presented in Table 9 may perhaps be explained by the fact that pyrethrins are less effective or slower in action in sprays containing heavy oil as shown by Wigglesworth (1942).

TABLE 3. *Kill of* Aëdes aegypti *exposed to dyed insecticidal mists with and without non-volatile matter added to increase the particle size. The figures represent averages for males and females. Two tests on about* 100 *insects were carried out*

Spray	Average dose of pyrethrins accumulated (mg./kg.)	Average % kill in 24 hr.
Without lubricating oil	1·5	45
With lubricating oil	3·5	64

The behaviour of the insect in relation to the dose accumulated

The important part which the flight activity of the insect plays in determining the dose of insecticide accumulated has been fully described (David & Bracey, 1946). It was observed that amputating the tarsal joints of *Aëdes* apparently increased the flight activity, since insects could no longer alight on the ceiling or vertical walls of the cages when they were exposed to a spray mist. On the other hand, within an hour or so of a blood meal female *Aëdes* were less active than insects which had been fed on sugar and water in the normal way. These observations were confirmed by exposing normal females, females with their tarsal joints amputated, and blood-fed insects

to a dyed pyrethrum spray simultaneously and subsequently determining the quantity of dye accumulated and the percentage kill obtained (Table 4).

TABLE 4. *The quantity of Sudan III collected (in μg.) by 10 female* Aëdes aegypti *in the conditions indicated in column 1 when exposed to a dyed pyrethrum spray mist. The figures represent the average of three determinations on about thirty insects*

State of insects	Quantity of Sudan III (in μg.)	Average 24 hr. kill (%)
Normal	0·43	16
Tarsi removed	0·73	77
Blood fed	0·26	0

The distribution and fate of the accumulated spray

When insects are exposed to spray mists of particle diameter below about 10 μ, in which the flight movements of the insects are largely responsible for the accumulation of the insecticidal dose, much more insecticide is collected on the wings than on the rest of the body (David, 1945). The colorimetric method permits quantitative estimations of the dye collected on the wings and body to be made. Batches of insects were exposed to dyed spray mists and then chloroformed. The wings were amputated and colorimetric estimations were made of the quantity of Sudan III extracted from the bodies and wings.

(*a*) *Experiments on* Musca domestica

The experiments on *Musca domestica* were extended to show the subsequent fate of the dye collected. The insects which had been exposed to the dyed spray (which was not appreciably toxic) were divided into three groups. Those in the first group were extracted immediately after being exposed, while those in the second and third groups were not extracted until 2 and 24 hr. later respectively. During this time they were kept in a constant-temperature room and had ample opportunity to clean themselves.

TABLE 5. *The relative quantities of Sudan III collected on the bodies and wings of male and female houseflies exposed to a dyed spray mist. The figures represent μg. of Sudan III collected by five flies*

Time interval before extraction (hr.)	Sudan III recovered in μg.			
	Males		Females	
	On body	On wings	On body	On wings
0·25	0·6	1·7	0·6	2·2
2·0	0·4	0·8	0·4	0·8
24·0	0·0	0·0	0·0	0·0

It is apparent from Table 5 that the quantity of dye accumulated on the wings is 3–4 times that accumulated on the body of *Musca* during exposure to a fine spray mist, and that the dye subsequently disappears either by being absorbed directly or as a result of the cleaning activities of the flies. As shown above (Table 1) appreciable quantities of dye penetrate the integument of the living fly. However, the important part which the cleaning processes carried out by the flies play in removing the dyed spray can be readily demonstrated.

A batch of houseflies was exposed to a dyed spray mist of 1 % Sudan III in 50/50 mixture of benzene and odourless kerosene. After the exposure the insects were chloroformed and four batches of twenty females were selected and treated as follows:

Batch (*a*): Bodies and wings extracted separately immediately after the exposure.

TABLE 6. *Batches of five female* Musca domestica *sprayed with Sudan III solution showing distribution of dye between wings and body and loss of dye from wings by cleaning and penetration in living insects but no loss by penetration in dead insects*

Treatment of insects	Sudan III recovered in μg.	
	Body	Wings
Batch (*a*) Normal extracted immediately	2·0	6·0
Batch (*b*) Normal extracted 24 hr. later	0·0	0·0
Batch (*c*) Legless extracted 24 hr. later	0·0	3·0
Batch (*d*) Killed extracted 24 hr. later	1·0	6·0

Batch (*b*): The extractions made 18 hr. later. During the interval the insects recovered and cleaned themselves.

Batch (*c*): The extractions made 18 hr. later. Before recovery from the chloroform took place the mid- and hindlegs were amputated so that the insects could not clean their wings.

Batch (*d*): The extractions made 18 hr. later. Instead of being allowed to recover from the chloroform the insects were killed with ethyl formate vapour and kept.

A comparison of the quantity of dye present on groups (*a*) and (*b*) (Table 6) shows the decrease which takes place in 18 hr. in the normal living fly. When group (*c*) (in which no cleaning of the wings by the legs can take place) is considered in addition, it can be seen that dye disappears from the wings by absorption and translocation in the wing veins (it is later excreted), but that the total lost is not more than about half of that which occurs in normal flies. The addition of group (*d*) to the comparison shows that

absorption from the wing surface occurs only in the living fly—the relevant point being presumably the wing vein circulation (Thomsen, 1938).

In order to follow the absorption of Sudan III from the wing surface more closely than was possible with the previous technique houseflies were chilled, their mid- and hindlegs were amputated, and 1/20,000 c.c. of the dyed spray was applied to the wing distal to the anterior cross-vein. The use of chloroform which may alter the permeability of the wings was avoided (Beament, 1945). Dissection of the flies, which were individually caged after treatment to prevent mutual contamination, showed that Sudan III had reached the malpighian tubules in detectable amounts within 1 hr. at 28° C., while after 3 hr. it was abundant in the malpighian tubules and had occasionally reached the rectum.

(*b*) *Experiments on* Aëdes aegypti

The quantity of spray collected on the wings and body of *Aëdes* was measured by a similar procedure to that used with *Musca*. The results obtained are given in Table 7. It will be noted that a rather larger proportion, i.e. 4–6 times as much, of the total amount of spray collected was recovered from the wings than in the case of *Musca*, an observation which is in line with that already reported to the effect that *Musca* collects a large number of spray droplets on the abdomen while *Aëdes* does not.

TABLE 7. *The relative quantities of Sudan III collected on the bodies and wings of male and female* Aëdes aegypti *exposed to a dyed spray mist. The figures represent* μg. *Sudan III collected by ten insects*

Time interval before extraction (hr.)	Sudan III recovered in μg. Males: On body	Males: On wings	Females: On body	Females: On wings
0·25	0·1	0·4	0·1	0·6
24·0	0·0	0·1	0·0	0·2

THE IMPORTANCE OF THE INSECTICIDE ACCUMULATED ON THE WINGS OF *MUSCA* AND *AËDES*

When an insect is struck by a moving spray droplet, contact may occur at random on any part of the body, but under the circumstances considered here, where the flight movements of the insects are responsible for the impacts, the insecticidal dose is distributed in a more or less definite way between the body and the wings.

It is not difficult to demonstrate the importance of the insecticide collected on the wings. Groups of insects were exposed to a spray mist of D.D.T. in odourless kerosene containing 5 % of lubricating oil (in this case medicinal paraffin), and after the exposure the insects were divided into two groups. During the following 10 min. the wings were removed from the insects in one group. Both batches were then transferred to a constant-temperature room and the percentage killed was estimated 18–24 hr. later (Table 8).

TABLE 8. *The reduction in 24 hr. percentage kill of female* Aëdes *and* Musca *produced by amputating the wings immediately after exposure to a spray mist, in comparison with unoperated controls. The average figures are based on five and six tests respectively with about 20–30 insects per test*

Condition of insects	*Aëdes aegypti*: Average kill (%)	*Aëdes aegypti*: Average angle (±1·7)	*Musca domestica*: Average kill (%)	*Musca domestica*: Average angle (±1·7)
Normal	87	69·7	82	66
Wings removed	35	35·3	36	36

The results given in Table 8 show clearly the importance of the insecticide collected on the wings under circumstances in which the flight movements of the insects are responsible for the impact with the spray droplets. Evidence has previously been presented that Sudan III may penetrate directly into the wings and be carried by the circulation to the body cavity or it may be transferred to the legs during cleaning and penetrate from there. More recent incomplete observations show that certain insecticides, e.g. D.D.T., may also penetrate slowly directly from the wings.

THE APPROXIMATE SURFACE MEDIAN LETHAL DOSES OF VARIOUS INSECTICIDES TO *MUSCA DOMESTICA* AND *AËDES AEGYPTI*

The general equipment and methods already described have been employed in order to determine what might be called the surface median lethal doses of pyrethrins, 2:2-bis (*para*chlorophenyl)-1:1:1-trichlorethane (D.D.T.; the pure compound was employed) and pure hexachloro*cyclo*hexane (666) to *Aëdes* and *Musca*. At the time of the tests the mosquitoes were between 1 and 4 days old and the houseflies between 3 and 5 days old. The results obtained necessarily represent only approximate maximum figures, since little is known concerning the percentage of material collected on the body and wings during flight which actually reaches a site of effective action within the insect. Although much of the spray originally collected will be lost to the surroundings or fail to reach a site of effective action, the values obtained should approximately represent

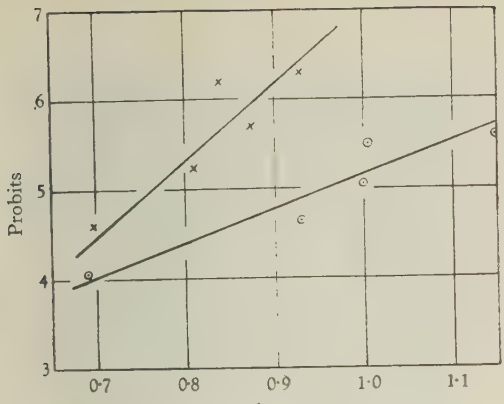

Fig. 1 *a*.

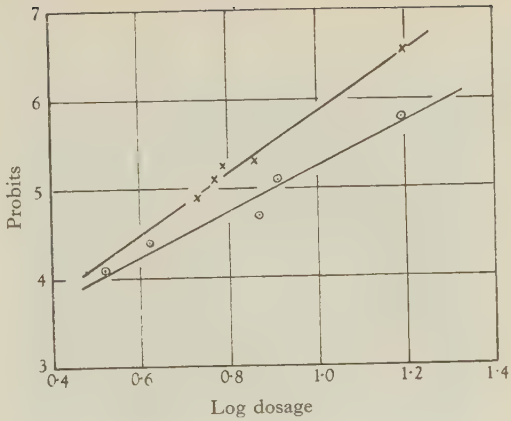

Fig. 2 *a*.

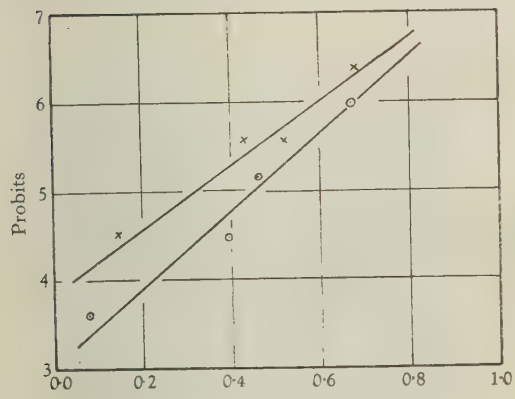

Fig. 1 *b*.

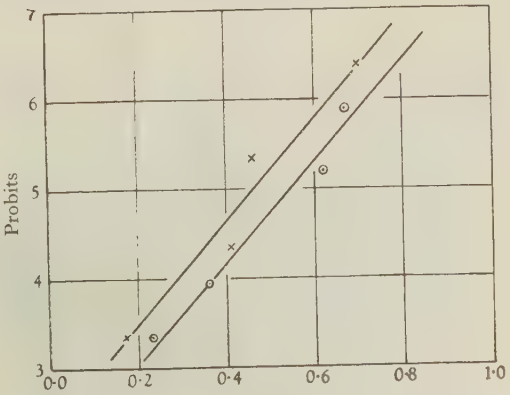

Fig. 2 *b*.

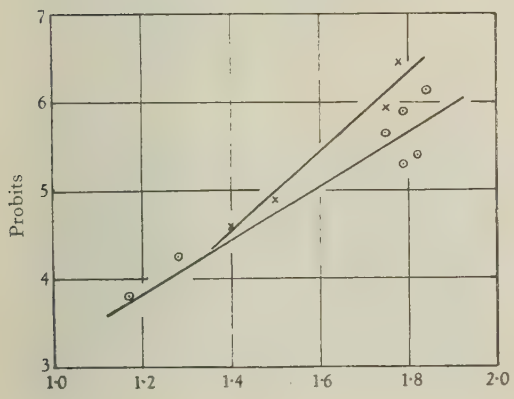

Fig. 1 *c*.

Text-Figs. 1 *a*, *b* and *c*. Provisional probit-log-dosage regression lines for (*a*) D.D.T., (*b*) 666, and (*c*) pyrethrins applied to male (×) and female (⊙) *Musca domestica* 3–5 days old.

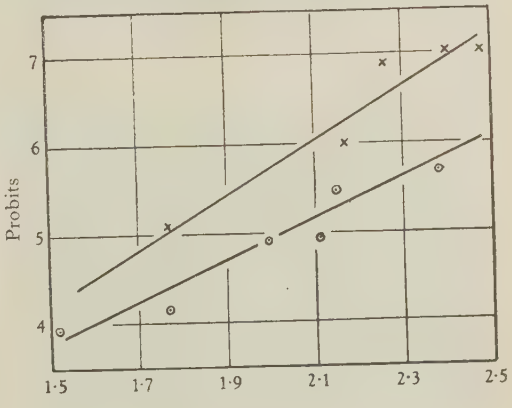

Fig. 2 *c*.

Text-Figs. 2 *a*, *b* and *c*. Provisional probit-log-dosage regression lines for (*a*) D.D.T., (*b*) 666, and (*c*) pyrethrins applied to male (×) and female (⊙) *Aëdes aegypti* 1–4 days old.

the quantities which have to be collected during an exposure in order that a dose lethal to 50% of the insects treated will penetrate to the desired locations.

The spray solutions employed consisted of 0·3% w/v of D.D.T. or of 666 and 1% Sudan III dissolved in a 50/50 mixture of odourless distillate and benzene. These solutions were used in the tests with both *Musca* and *Aëdes*. The pyrethrum sprays were also prepared with the same solvent mixture and contained 1% of Sudan III; the pyrethrins content was 0·1% w/v for *Aëdes* and 2·0 w/v for *Musca*.

These spray solutions were introduced into the chamber in the normal way. The dose accumulated by the test insects was varied by introducing the exposure cages into the chamber at various intervals after spraying. During the exposures the cages were shaken to promote uniform flight. Since the actual doses accumulated by the insects were measured, variations in the details of the testing procedure are unimportant, and are consequently not specified.

At the end of the test exposure the insects were divided into two groups. One group was retained for estimation of the kill 18–24 hr. later, while a colorimetric estimation of the dose accumulated was carried out on the remainder of the batch.

The results obtained are presented in the form of provisional regression lines in Text-figs. 1 *a*, *b* and *c* and 2 *a*, *b* and *c*, from which the approximate maximum median lethal dosages shown in Table 9 were read off.

TABLE 9. *The maximum median lethal dosages of three insecticides to* Musca (3–5 *days old*) *and* Aëdes (1–4 *days old*) *as determined by the colorimetric method and assessed from the provisional regression lines of Text-figs.* 1 *and* 2

	Maximum median lethal dose (mg./kg.)			
	Musca		*Aëdes*	
Insecticide	Males	Females	Males	Females
D.D.T. 0·3% w/v	6·0	9·0	5·5	8·0
666 0·3% w/v	2·0	3·0	3·0	3·5
Pyrethrins 0·1% w/v	—	—	0·5	1·0
Pyrethrins 2·0% w/v	31·0	38·0	—	—

It has already been explained that the colorimetric method merely measures the dose collected which leads to a 50% kill without any indication of the percentage of poison which reaches a site of effective action. The labour of calculating the position of the regression lines therefore hardly seemed justifiable, and in any case the resistance of both *Musca* and *Aëdes* to poisons is known to vary. This is illustrated by the less precisely determined values which were obtained in the course of the preliminary and confirmatory work carried out in connexion with the experiments presented in Text-figs. 1 *a*, *b* and *c* and 2 *a*, *b* and *c*.

Discussion and comments

It has been shown that when exposed to finely divided mists composed of slowly moving particles mainly below 10 μ in diameter, insects accumulate a high percentage of the spray on their wings. It is to be expected that, under practical conditions, or in the Peet-Grady chamber where the insects may be flying during the time when the spray is applied, the result would not be the same, since the insects might be struck at random by rapidly moving spray particles. Under such circumstances a high percentage of the effective dosage might land directly on the body. Whether this is so or not could be readily determined by means of the colorimetric method which has been described.

TABLE 10. *The range of maximum median lethal dosage values observed in the course of the preparatory and confirmatory work in connexion with the more precisely determined results given in Table* 9

	Maximum median lethal doses (mg./kg.)			
	Musca		*Aëdes*	
Insecticide	Males	Females	Males	Females
D.D.T. 0·3% w/v	5·5–7·0	9·3–10·5	4·5–5·5	7·5–8·5
666 0·3% w/v	2	3	2·0–2·5	2·5–3·5
Pyrethrins 0·1% w/v	—	—	0·5–1·0	1·0–1·5
Pyrethrins 2·0% w/v	30–35	40	—	—

The colorimetric technique of estimating the quantity of spray actually accumulated by a test group of insects should also prove useful in other directions. For example, it could be used to correlate the results obtained in different types of chambers and to investigate the efficiency of atomizers and the effects of changes in the spraying technique and exposure conditions. Again, whenever the behaviour of the insect modifies the dose accumulated and the behaviour is in turn influenced by the nature of the toxic agent this interrelationship could also be investigated.

This investigation has been carried out as a part of the programme of the Agricultural Research Council Unit of Insect Physiology.

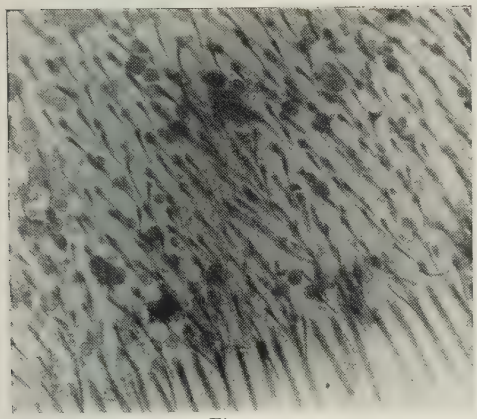

Fig. 1 *a*

Fig. 1 *b*

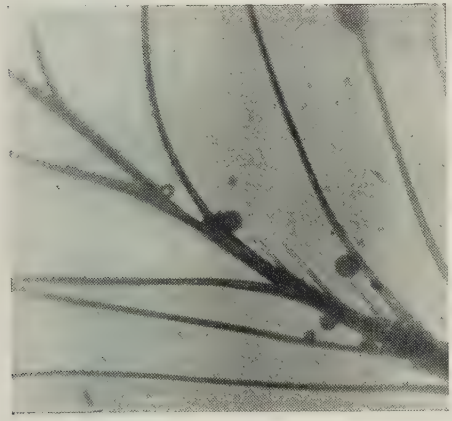

Fig. 2

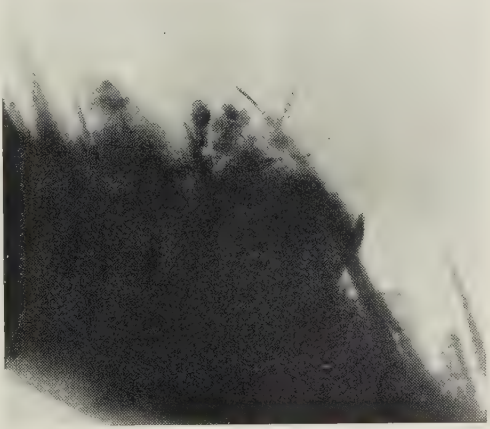

Fig. 3

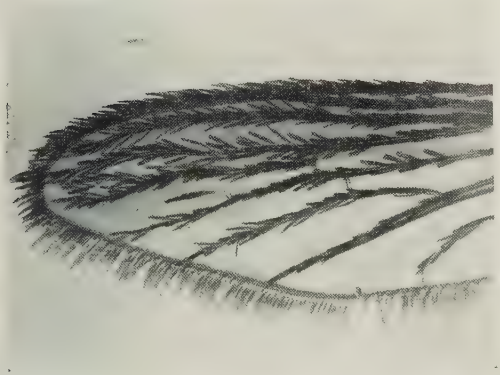

Fig. 4 *a*

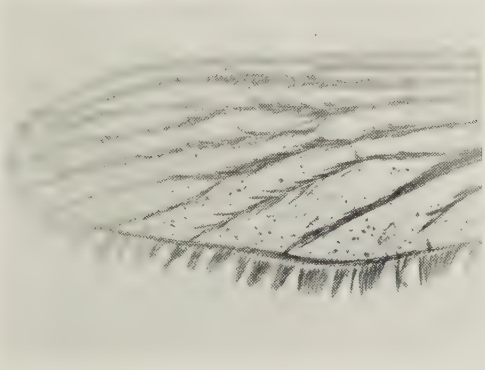

Fig. 4 *b*

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EXPLANATION OF PLATE 9

Figs. 1*a*, *b*. (*a*) Aqueous droplets on the wing of *Musca domestica* L. collected during flight. Note the regular outline in comparison with the oil spray droplets shown in (*b*).

Fig. 2. Aqueous spray droplets collected on the antennae of *Musca* during flight.

Fig. 3. The first tarsal joint of the hindleg of *Musca* showing aqueous droplets held among the bristles. These droplets have been combed from the wing.

Figs. 4*a*, *b*. Showing wings of *Aëdes* which have been exposed to (*a*) a spray of Sudan III in odourless distillate and (*b*) the same spray containing 5 % of added non-volatile lubricating oil.

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Effects of atmospheric environment, before and after treatment, on the toxicity to insects of contact poisons. I

By C. Potter and E. M. Gillham,
Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden

(With 7 Text-figures)

Experiments were made on the effect of conditions before and after treatment on the toxicity to adult *Tribolium castaneum* Hbst. of the following contact poisons in the media stated: (1) pyrethrins, (2) lauryl thiocyanate, (3) nicotine—all in aqueous medium, (4) dinitro-*o*-cresol in ethylene glycol, (5) Wakefield half-white oil, (6) D.D.T. in Wakefield half-white oil.

The difference in environment before spraying did not have any marked effect but, with the exceptions of nicotine and petroleum oil, all the toxicants used were more insecticidal when the beetles were kept under cool conditions after spraying. Nicotine showed little difference due to after-treatment when an inverted filter funnel was used to confine the insects, but seemed markedly more toxic under cooler conditions of after-treatment when the insects were confined in the dishes by means of muslin.

Wakefield half-white oil, a non-volatile petroleum oil, proved more toxic when the insects were kept under warm conditions after treatment than under cool conditions. The increase in toxicity of chemically active contact poisons under cool conditions of after-treatment appeared to occur whatever the nature of the carrier, whether volatile or non-volatile, water or oil.

In the substances tested, with the exception of nicotine under special circumstances, the increase in toxicity under cool conditions of after-treatment occurred whatever the volatility of the poison.

The change in toxicity, when cool conditions were compared with hot, varied with the poison used. With nicotine in aqueous medium the change was relatively small, the toxicity under cold conditions throughout being 1·23 times the toxicity under hot conditions throughout. At the other extreme with pyrethrins and terpineol in aqueous medium there was a large alteration, the toxicity under cold conditions throughout being about 7 times the toxicity under the hot conditions throughout.

Introduction

The number and nature of the factors affecting the toxicity of poisons to insects are probably not completely known, and the amount of quantitative data on their effect is meagre. The conventional procedure is to control and specify as many as possible of the conditions of the test, and to trust that no important factor has been omitted or left uncontrolled. This renders it impossible to determine how far the results of any given experiments are generally applicable, since they may apply under only one set of conditions, i.e. those of the experiment. Further investigation is therefore needed into the factors affecting toxicity and their relative importance.

During the course of some work on the activation of the pyrethrins by various substances, it was found that while in one experiment a given substance would show an activating effect, in another it would not. It was thought that this might be due to variations in the conditions of the different experiments (e.g. in temperature and humidity), and a further test was planned to obtain information on this point by varying the conditions before and after treatment. In the absence of adequate facilities for controlling temperature and humidity, contrasted conditions were obtained by using widely different temperatures. It must be recognized, however, that any effect produced may not be *directly* due to temperature.

An experiment, the results of which were confirmed by a similar one carried out later, strongly indicated that although the differing conditions did not necessarily influence activating effect, they did clearly affect toxicity. This led to experiments with different insecticides to find whether this marked effect was specific to the pyrethrins, and to obtain further information on its applicability.

Only one species of insect was used, and this severely limits the deductions that may be drawn from this work, but it may serve as a starting-point for more detailed investigations. Owing to the number of factors involved, the statistical treatment of the results of the early experiments was rather

more complex than that employed in the usual estimation of relative toxicity. The method used is the subject of a separate communication by D. J. Finney (Finney, 1946).

PREVIOUS WORK

The important distinction between effects produced by the temperature and humidity conditions during treatment and those produced by these conditions before and after treatment does not always appear to have been made by previous investigators.

The effect of temperature and humidity on the toxicity of fumigants has been discussed by Cotton (1943). The work done is primarily on their effect during exposure to the fumigant, and so far no generally applicable rule has been formulated. Some evidence shows that the temperature at which the test insects are maintained before treatment affects toxicity, but none is given on the effect of keeping conditions after treatment.

Ellisor & Blair (1940), working on the effect of temperature on the toxicity of stomach poisons, established the median lethal doses of synthetic cryolite, acid lead arsenate, basic copper arsenate and calcium arsenate to the 5th stage larvae of *Anticarsia gemmatalis* Hb. and *Laphygma* (*Prodenia*) *eridania* Cram. at 60 and at 80° F. In all but one instance the toxicity was greater at 60 than at 80° F., but the mean survival period was shorter at 80° F.

Other workers' results concerning the effect of temperature and humidity conditions before and after treatment on the toxicity of contact poisons is summarized below. There is some evidence that an increase in temperature during treatment increases toxicity, but there appear to be no systematic experiments on its effect when other factors are kept constant. The effect of humidity during application has been little explored.

Gösswald (1933) investigated the action of a pyrethrum dust on a number of forest pests under different conditions of temperature and humidity. He concluded that any given species was most resistant at its specific 'vital optimum' temperature, i.e. the temperature that had been found most favourable for its development. Increase in humidity increased the resistance. Gösswald's data are complicated because in many experiments, not conducted at or near 'vital optimum' conditions, there is up to 100% mortality in the untreated insects, and no allowance appears to be made for this when assessing the toxicity of the poison.

Whitcomb (1934–6) experimented on the effect of temperature on the toxicity of various substances to greenhouse red spider, *Tetranychus telarius* L. He found that the toxicity varied greatly with the temperature when the test subjects were kept at 60 and 80° F. after treatment; the effectiveness of some materials varied directly with increase in temperature and others inversely. Of thirty-four unnamed materials tested sixteen were consistently more effective at 60 than at 80° F.

Heavy lubricating oil emulsions were more effective at high temperatures, light oil emulsions at 60° F. Soaps gave inconsistent results but were in general more effective at 60° F. Soluble sulphurs and lime sulphur were better at higher temperatures, but suspended sulphurs such as colloidal or wettable sulphur at 60° F. Pyrethrum extracts were less effective at higher temperatures, but extracts of derris showed increased effectiveness. This work gives very little actual experimental data.

Klinger (1936) investigating the insecticidal action of sprays of pyrethrum and derris on a variety of insects found that the toxicity varied with the after-treatment temperature, and he submits some evidence that the toxicity, presumably of both pyrethrum and derris, is decreased at higher temperatures 30–30·8° C. as contrasted with 14–15 and 19–20° C.

Böttcher (1938) found that pyrethrum was considerably more toxic to bees both as a stomach poison and as a contact poison when they were kept at 20° C. than when they were kept at 34·5° C. However, he found in earlier work that the reverse was true of nicotine. Apparently the poisons were applied in aqueous medium. In a later paper, Böttcher (1939) found that the toxicity of rotenone to bees both as a stomach poison and as a contact poison was lower at 34·5 than at 20° C., but that the difference was small.

Crauford-Benson (1938), using *Ahasverus advena* Waltl. adults as test subjects, a derris insecticide in aqueous medium and an immersion technique, found that where the insects were bred at 20° C. and 75% R.H. and returned to these conditions after treatment they were less resistant to the insecticide than when they were bred at 25° C. and 75% R.H. and put back under these conditions after treatment. This was found with several different immersion temperatures. It is noteworthy that 25° C. and 75% R.H. were the optimum conditions for rearing the insect. Crauford-Benson also worked on the effect of different humidities before and after treatment, and found that a variation of between 55 and 95% R.H. had a slight effect. Decreasing the humidity increased the toxicity of the poison.

Eagleson (1942) studied the effect of different temperatures on the recovery of houseflies treated with the pyrethrins and lethane (β-butoxy-β'-thiocyanodiethylether), atomized in solution in refined kerosene. In this work the humidity was standardized at 50% R.H. He found increased recovery of the flies with both insecticides as the temperature was increased from 22 to 38° C.; the recovery gradient for the pyrethrins was, however, different from that of lethane.

Harrison & Allen (1943) using turnip aphis noted that pine-tar soap spray gave 90% control in conditions of high humidity and low temperature, but that nicotine sulphate had to be added to obtain a satisfactory kill under conditions of low humidity and high temperature.

Lindquist *et al.* (1944) found that when houseflies were exposed to films of D.D.T. and then divided up into batches after treatment, the different batches being kept at 70, 90 and 100° F., there was a greater recovery at the higher temperature than at the lower.

The work referred to above consisted of observations made in the laboratory where the insects were kept after treatment, and in some instances before treatment, either under controlled conditions or conditions that were stated.

It is difficult to deduce any general principles from the work outlined above. In several instances, and with a number of toxic principles, an increase in temperature after treatment favours recovery, but the reverse has been recorded, and it seems that the result may vary with the insect and the insecticide. The theory that the insect is most resistant when kept after treatment under the conditions that are the optimum for rearing that particular species appears to be worthy of further investigation. The work described in this paper aims at providing some quantitative data which may serve as a starting point for a more detailed study.

Materials and method

The insects used were adult *Tribolium castaneum* Hbst., reared on wholemeal flour at 27° C. and approximately 60% R.H. The method of rearing has already been described (Tattersfield & Potter, 1943). The insects were treated in a spraying apparatus already described (Potter, 1941), which is designed to give an even deposit over the sprayed area and to provide a wide range of deposits.

The full range of treatments consisted of various combinations of four conditions: (1) Hot before treatment, the insects being kept in the constant-temperature room at 80° F. and approximately 60% R.H. until treated. (2) Cold before treatment, which involved leaving them in the constant-temperature room at 80° F. and approximately 60% R.H. until 1 hr. before spraying, when they were placed in a cool incubator at approximately 55° F. (3) Hot after treatment, the insects being returned after spraying to the room at 80° F. and approximately 60% R.H. (4) Cold after spraying, the insects being taken to a basement room at approximately 60° F. and a variable humidity.

In the absence of accurate constant-temperature facilities a 20° F. difference was made between the main hot treatment and the main cold treatment. Control of humidity throughout was not possible. The hot room was fitted with humidity control but even so, there was considerable variation and there was no control in the cool room.

Measurements were taken by means of a whirling hygrometer, and these are given in Table 1. They indicate that the relative humidity was generally somewhat lower in the cool room than in the hot room. Table 1 gives the data for the spraying conditions for all the experiments described.

All the evidence acquired indicated that temperature was the overriding factor within the limits of temperature and humidity occurring in this series of experiments, and it seems probable that the absence of humidity control has not significantly affected the results, although extreme humidities, particularly high, may have an important influence on toxicity.

Insects were left for a varying length of time after treatment with the different insecticides, but all were inspected on a constant-temperature warm plate which has been previously described (Tattersfield & Potter, 1943).

The two experiments first described, although carried out with an interval of 2½ years, both deal with the effect of variation of the conditions before and after treatment on the toxicity of the pyrethrins and of pyrethrins plus terpineol; they are therefore described together in order that they may be more easily compared.

Exps. I and II. The effect of differences in the environment before and after spraying on the toxicity to adult *Tribolium castaneum* Hbst., of pyrethrins and pyrethrins + terpineol in aqueous medium

Exp. I. 29 *October* 1942

Spraying conditions (see Table 1). Three replicas each of approximately twenty insects for each dilution.

Keeping conditions. H.H. = hot room—treatment—hot room. H.C. = hot room—treatment—cool room. C.H. = hot room—cooled at 55–57° F., an hour before treatment commences—treatment—hot room. C.C. = hot room—cooled at 55–57° F. an hour before treatment commences—treatment—cool room.

Procedure and general data

On the day of spraying the insects were put in 2 × 1 in. glass tubes, twenty per tube. These were kept in the hot room until the experiment commenced in the afternoon. Insects for H.H. treatment were kept in the hot room until sprayed, one tube at a time being brought out, the insects sprayed, and immediately returned to the hot room and left. H.C. insects were kept in the hot room until treatment,

TABLE 1. *Summarized data for spraying and keeping conditions. Potter apparatus with 1·7 in. gap*

	Spraying conditions										Keeping conditions				
											Temp. °F.			% R.H.	
Exp. no.	Pressure (cm.Hg)	Spray in reservoir (c.c.)	Temp. °F. Start	Temp. °F. End	% R.H. Start	% R.H. End	Insecticide	Medium	Deposit wt. (mg./cm.²) Start	Deposit wt. (mg./cm.²) End	Outside air	Hot room	Cool room	Hot room	Cool room
I	18	5	65	67	60	60	Pyrethrins	10 % acetone + 0·5 % saponin	7·9, 8·3, 7·6, 7·6, 7·8, 7·4	8·0, 8·1	48	80	60 (approx.)	61‡ and 56†	58‡ and 63†
								As above + 1 % terpineol		7·2, 7·1, 7·4					
II	27·6 / 19·4	5	75	75	55	48	Pyrethrins	15 % acetone + 0·5 % saponin	6·9, 7·1, 7·2, 7·1	6·8, 6·8	62–67*	80	60–62	60	48–56
								As above + 1 % terpineol	7·0, 6·8	7·1, 6·6, 6·9					
III	18	4	56	68·5	58	53	Lauryl thiocyanate	20 % acetone in 0·1 % s. lorol	4·9, 4·8, 5·0	5·0, 5·0, 5·0	45	80·6	56–59	52‡	50‡
IV	18	5	65	67	54	59	Nicotine	20 % acetone in 0·1 % s. lorol	6·3, 6·3	6·7, 6·5	44–49	80·6	58–60	64‡	62‡
V	20	5	66	—	50	—	Nicotine	20 % acetone in 0·1 % s. lorol	5·9, 6·3, 6·3	6·3, 6·3	44	80	57–66	57‡	45‡
VI	18	1	65	68	52	58	Dinitro-*o*-cresol	Ethylene glycol	1·5, 1·7, 1·7	1·6, 1·9, 1·6	45–50	80·6	58–60	61‡	36‡
VII	53	1 / 1·5 / 2·0 / 2·5 / 3·0 / 3·5	68	—	63	—	White oil			0·63, 0·63 / 0·9, 0·97 / 1·11, 1·06 / 1·41, 1·3, 1·39 / 1·65, 1·67 / 1·97, 1·95	59	80	58	—	—
VIII	31·6–37·8	4	80	79	68	57	D.D.T.	Half-white oil		13·2, 13·2	—	80	65	—	—

* R.H. 53–61 %. † Records by whirling hygrometer morning after spraying. ‡ Records by whirling hygrometer immediately after spraying.

when they were brought out one tube at a time and the insects sprayed. After spraying they were put on a tray outside the laboratory until spraying was complete, when they were conveyed to the cool room and left until inspected. The tubes containing the C.H. insects were put in a cool incubator at 55–57° F. 1 hr. before spraying commenced. They were brought out one by one and sprayed, then taken directly to the hot room and left until inspected. C.C. insects were put in the cool incubator at 55–57° F. 1 hr. before spraying commenced, taken out one tube at a time and sprayed, after which they were put on a tray outside until the end of the spraying. They were then taken down to the cool room and left until inspected.

Procedure and general data

The insects were taken from the cultures on the morning of spraying. Eight cultures, started on different dates, were used. The insects from the eight cultures were mixed in a battery jar and then insects from the jar were put into 2 × 1 in. glass tubes in batches of twenty. At 4.30 p.m. of the same day half of the insects were put into a cool incubator at 55–57° F., and the rest put back into the constant-temperature room at 80° F. The R.H. of the cool incubator just before spraying was between 60 and 70%. Treatment commenced at 4.30 p.m. the following day and ended at 6.30 p.m. The procedure for treatment was as in Exp. I, except that the insects

TABLE 2. *Results of Exp. I*

Solutions: (*a*) pyrethrins in 10% v/v acetone and 0·5% w/v saponin;
(*b*) pyrethrins + 1% v/v terpineol in 10% v/v acetone and 0·5% w/v saponin.

Conc. pyrethrins w/v									
Pyrethrin I %	Pyrethrin II %	Mortality H.H.	Kill %* H.H.	Mortality H.C.	Kill %* H.C.	Mortality C.H.	Kill %* C.H.	Mortality C.C.	Kill %* C.C.
				Solution (*a*) pyrethrins					
0·015	0·0045	39/44	89	75/75	100	63/70	90	80/80	100
0·010	0·0030	28/50	55	65/72	90	56/72	78	79/80	99
0·005	0·0015	8/60	11	48/63	76	16/70	21	61/85	72
Sprayed control		1/41	—	1/70	—	1/60	—	0/73	—
				Solution (*b*) pyrethrins + 1 % v/v terpineol					
0·015	0·0045	37/63	59	70/70	100	60/76	79	74/74	100
0·010	0·0030	10/76	13	45/49	92	34/75	44	69/70	99
0·005	0·0015	3/68	4	47/60	78	16/71	22	65/69	94
Sprayed control		0/68	—	1/72	—	1/75	—	2/58	—

* Corrected percentage allowing for mortality in the controls.

The insects were all sprayed in 9 cm. Petri dishes containing a circle of tricoline in the bottom. After treatment they were covered with an inverted filter funnel, which confined them on the tricoline circle and were left in this manner until inspected. Details of the keeping conditions are given in Table 1.

The insects were inspected on a warm plate (37·5–40° C.) on the 4th, and again on the 8th day after treatment: the results are taken from the first inspection but there was no essential difference between the two sets of results.

Exp. II. 23 March 1945

Spraying conditions (see Table 1). Three replicas each of approximately twenty insects for each dilution.

Keeping conditions. As in Exp. I but pre-treatment cooling for 24 hr.

that were to be kept under cool-storage conditions were taken to the cool room as each set of replicates was finished. Details of keeping conditions are given in Table 1.

The insects were inspected on the warm plate on the 4th day after spraying, and again on the 12th day. The results are taken from the first inspection since there was some mortality in the controls on the second inspection, but apart from the slightly increased mortality there did not appear to be any significant difference between the two.

General results of analysis of exps. I and II

The data obtained in both experiments were subjected to statistical analysis and the curves derived from the data are shown in Figs. 1 and 2. Both experiments show that the conditions under which the insects are kept greatly affects the toxicity of the

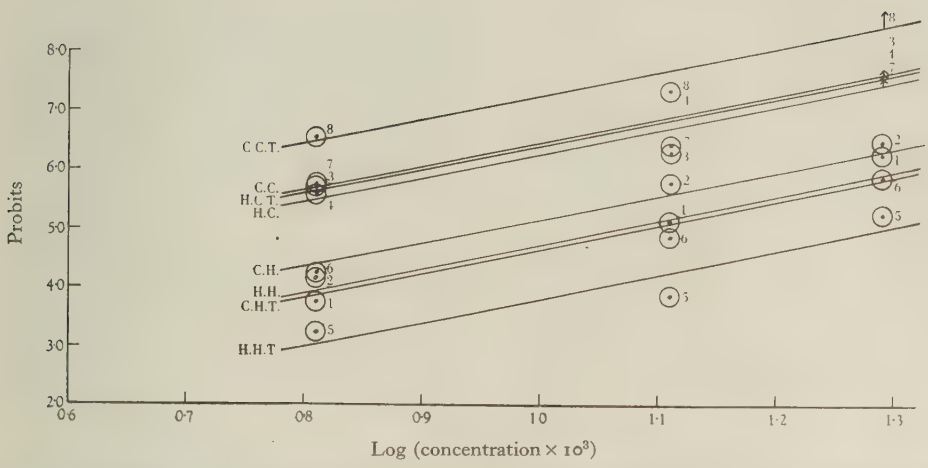

Fig. 1. Graph showing the effect of differences in the environment before and after spraying on the toxicity to adult *Tribolium castaneum* Hbst., of pyrethrins and pyrethrins + terpineol in aqueous medium. (Experiment I.) Graph shows the relations between probit mortality and log concentration after constraining the lines to be parallel. ↑ = dose at which 100% kill occurred.

		Regression equations	
1 = H.H. = Hot before treatment, hot after treatment	without terpineol	H.H.	$Y = 1{\cdot}15 + 3{\cdot}65x$
2 = C.H. = Cool before treatment, hot after treatment	without terpineol	C.H.	$Y = 1{\cdot}51 + 3{\cdot}65x$
3 = H.C. = Hot before treatment, cool after treatment	without terpineol	H.C.	$Y = 2{\cdot}53 + 3{\cdot}65x$
4 = C.C. = Cool before treatment, cool after treatment	without terpineol	C.C.	$Y = 2{\cdot}74 + 3{\cdot}65x$
5 = H.H.T. = Hot before treatment, hot after treatment	with terpineol	H.H.T.	$Y = 0{\cdot}23 + 3{\cdot}65x$
6 = C.H.T. = Cool before treatment, hot after treatment	with terpineol	C.H.T.	$Y = 1{\cdot}02 + 3{\cdot}65x$
7 = H.C.T. = Hot before treatment, cool after treatment	with terpineol	H.C.T.	$Y = 2{\cdot}70 + 3{\cdot}65x$
8 = C.C.T. = Cool before treatment, cool after treatment	with terpineol	C.C.T.	$Y = 3{\cdot}50 + 3{\cdot}65x$

TABLE 3. *Results of Exp. II*

Solutions: (*a*) pyrethrins in 15% v/v acetone and 0·5% w/v saponin;
(*b*) pyrethrins + 1% v/v terpineol in 15% v/v acetone and 0·5% w/v saponin.

Conc. pyrethrins w/v									
Pyrethrin I %	Pyrethrin II %	Mortality H.H.	Kill %* H.H.	Mortality H.C.	Kill %* H.C.	Mortality C.H.	Kill %* C.H.	Mortality C.C.	Kill %* C.C.
Solution (*a*) pyrethrins									
0·0142	0·0137	47/62	76	59/59	100	48/55	87	61/61	100
0·0106	0·0103	37/59	63	58/58	100	54/57	95	60/60	100
0·0071	0·00685	33/57	58	58/58	100	49/60	82	60/60	100
0·00355	0·00342	5/59	8	55/60	92	14/56	25	52/59	88
0·00177	0·00171	2/59	3	28/59	47	2/57	4	38/65	58
Sprayed control		2/56	—	0/54	—	1/58	—	0/62	—
Solution (*b*) pyrethrins + 1% v/v terpineol									
0·0142	0·0137	55/58	95	58/58	100	59/60	98	61/61	100
0·0106	0·0103	52/63	83	60/60	100	55/60	92	60/60	100
0·0071	0·00685	26/59	44	61/61	100	28/58	48	62/62	100
0·00355	0·00342	8/59	14	55/56	98	15/58	26	59/60	98
0·00177	0·00171	6/58	10	51/59	86	2/55	4	50/58	86
Sprayed control		0/59	—	0/60	—	0/56	—	0/58	—

* Corrected percentage allowing for mortality in the controls.

pyrethrins, and that the difference is most marked between insects kept in the hot and those kept in the cool room *after* treatment.

It was found in the first experiment that the toxicity of the insecticide to the insects kept in the cool room after treatment averaged over the hot and cool pre-treatment, both with and without terpineol, was 3·16 times its toxicity when the insects were kept in the warm room after treatment; the corresponding figure for the second experiment was 4·99.

treatment may be varied within wide limits, in this instance between 1 hr. and 24 hr., without producing any large differences in toxicity. The differences in absolute toxicity between the two experiments may be explained, first by the different condition of the two sets of test insects, and secondly by a difference in the relative amounts of pyrethrin I and pyrethrin II in the two spraying solutions. These differences in no way affect the conclusions to be drawn from the experiment.

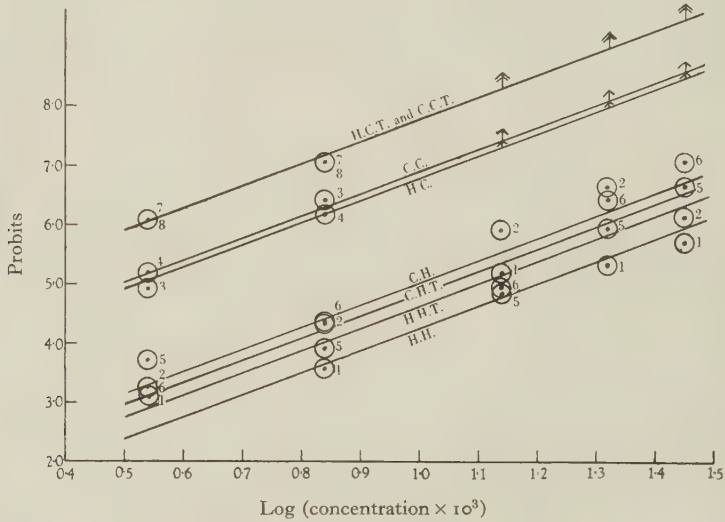

Fig. 2. Graph showing the effect of differences in the environment before and after spraying on the toxicity to adult *Tribolium castaneum* Hbst. of pyrethrins and pyrethrins + terpineol in aqueous medium. (Experiment II.) Graph shows the relation between probit mortality and log concentration after constraining the lines to be parallel. ↑ dose at which 100% kill occurred.

Regression equations

1 = H.H. $Y = 0{\cdot}53 + 3{\cdot}75x$	5 = H.H.T. $Y = 0{\cdot}89 + 3{\cdot}75x$
2 = C.H. $Y = 1{\cdot}29 + 3{\cdot}75x$	6 = C.H.T. $Y = 1{\cdot}09 + 3{\cdot}75x$
3 = H.C. $Y = 3{\cdot}04 + 3{\cdot}75x$	7 = H.C.T. $Y = 4{\cdot}03 + 3{\cdot}75x$
4 = C.C. $Y = 3{\cdot}15 + 3{\cdot}75x$	8 = C.C.T. $Y = 4{\cdot}03 + 3{\cdot}75x$

The effect of the addition of terpineol varied with the type of treatment and also in the two experiments; there was, however, a greater average difference in toxicity produced between cold and hot conditions of treatment when terpineol was added. In the first experiment pyrethrins + terpineol were 7·21 times as toxic under cool conditions than under hot, whereas the pyrethrins alone were 2·61 times as toxic when cool conditions were compared with hot, the corresponding figures for the second experiment being 6·90 and 5·01. The L.D. 50's from which these figures were derived are set out in Table 4.

A comparison of the two experiments indicates that the duration of the period of cooling before

Detailed results of analysis of Exps. I and II

When the standard methods of probit analysis were applied to the data in both experiments, each set of data was found to be satisfactorily fitted by a set of eight parallel probit regression lines (Figs. 1, 2). Unfortunately, in both experiments departures from these lines indicate heterogeneity in the data; there was, however, no indication of departure from parallelism.

The estimated median lethal doses are given in Table 4.

The effect of cold storage before spraying, counting the experiments with and without terpineol, averaging

over hot and cold conditions after spraying, was to decrease the log L.D. 50 by $0{\cdot}142 \pm 0{\cdot}034$ and $0{\cdot}072 \pm 0{\cdot}033$ respectively in the two experiments; these decreases are both significant, and represent relative potencies of 1·39 and 1·18 respectively. The corresponding effects of cold storage after spraying, counting the experiments with and without terpineol, averaging over hot and cold conditions before spraying, were much greater, being decreases of $0{\cdot}500 \pm 0{\cdot}046$ in the log L.D. 50 in the first experiment, $0{\cdot}698 \pm 0{\cdot}036$ in the second. These values represent relative potencies of 3·16 and 4·99 respectively. There was little indication that the magnitude of the effect of storage conditions after spraying was influenced by storage conditions before spraying; the measures of the interaction were $0{\cdot}012 \pm 0{\cdot}036$ and $0{\cdot}058 \pm 0{\cdot}033$ in the two experiments (Finney, 1946). The results, counting experiments with and without terpineol, show a total increase in toxicity for cold storage, both before and after spraying, over hot storage at both stages, amounting to 4·40 times in the first experiment and 5·89 times in the second. The estimated median lethal doses for the different combinations of storage conditions, averaged over tests with and without terpineol, are summarized in Table 5.

The conclusions just discussed represent averages of results with and without terpineol. The effect of addition of terpineol to the spray has been inconsistent. In the first experiment it increased the log L.D. 50 by $0{\cdot}029 \pm 0{\cdot}036$, an effect which was not significant, but which represented a reduction of 6% in the toxicity; in the second experiment terpineol decreased the log L.D. 50 by $0{\cdot}136 \pm 0{\cdot}034$, corresponding to a relative potency of 1·37. Mean values of the median lethal dose for various combinations of terpineol and storage conditions are summarized in Tables 6 and 7.

TABLE 4. *Median lethal doses of pyrethrins and pyrethrins plus terpineol to adult* Tribolium castaneum *Hbst. under varying conditions*

Treatment				L.D. 50% w/v total pyr.	
Pre-spraying	Post-spraying	Terpineol + or −	Symbol	Exp. I	Exp. II
Cold	Cold	−	C.C.	0·0044	0·0031
Hot	Cold	−	H.C.	0·0049	0·0033
Cold	Hot	−	C.H.	0·0092	0·0098
Hot	Hot	−	H.H.	0·0116	0·0156
Cold	Cold	+	C.C.T.	0·0027	0·0018
Hot	Cold	+	H.C.T.	0·0045	0·0018
Cold	Hot	+	C.H.T.	0·0122	0·0110
Hot	Hot	+	H.H.T.	0·0198	0·0125

TABLE 5. *The effect of storage conditions before and after spraying on the mean values of the* L.D. 50 *of pyrethrins* (% *w/v total pyrethrins*) *to adult* Tribolium castaneum *Hbst.*

(Results are averaged for tests with and without terpineol.)

Before spraying	After spraying: Cold	Hot	Mean*
	Exp. I		
Cold	0·0035	0·0106	0·0061
Hot	0·0047	0·0152	0·0084
Mean	0·0040	0·0127	0·0071
	Exp. II		
Cold	0·0024	0·0104	0·0050
Hot	0·0025	0·0140	0·0059
Mean	0·0024	0·0120	0·0054

* The means in this table have been obtained logarithmically, and appear as geometric *not* arithmetic means.

TABLE 6. *The effect of storage conditions before spraying and of terpineol on the mean values of the* L.D. 50 *of pyrethrins* (% *w/v total pyrethrins*) *to adult* Tribolium castaneum *Hbst.*

(Results are averaged over alternative storage conditions after spraying.)

Before spraying	Addition of terpineol to spray: Absent	Present	Mean*
	Exp. I		
Cold	0·0063	0·0058	0·0061
Hot	0·0075	0·0094	0·0084
Mean	0·0069	0·0084	0·0071
	Exp. II		
Cold	0·0055	0·0045	0·0050
Hot	0·0072	0·0048	0·0059
Mean	0·0063	0·0046	0·0054

* The means in this table have been obtained logarithmically, and appear as geometric *not* arithmetic means.

TABLE 7. *The effect of storage conditions after spraying and of terpineol on the mean values of the* L.D. 50 *of pyrethrins* (% *w/v total pyrethrins*) *to adult* Tribolium castaneum *Hbst.*

(Results are averaged over alternative storage conditions before spraying.)

Before spraying	Addition of terpineol to spray: Absent	Present	Mean*
	Exp. I		
Cold	0·0046	0·0035	0·0040
Hot	0·0104	0·0156	0·0127
Mean	0·0069	0·0074	0·0071
	Exp. II		
Cold	0·0032	0·0018	0·0024
Hot	0·0124	0·0118	0·0120
Mean	0·0063	0·0046	0·0054

* The means in this table have been obtained logarithmically, and appear as geometric *not* arithmetic means.

after spraying in increasing the beneficial effect of terpineol on potency; in the first experiment the addition of terpineol reduced the potency for hot conditions after spraying, increased it for cold, and in the second experiment the addition had practically no effect under hot conditions, but gave a 78% increase in potency under cold conditions after spraying.

It appears from these two experiments that the temperature at which the insects were kept before treatment, at any rate up to 24 hr. before treatment, can be varied within wide limits (55–80° F.) without greatly affecting subsequent toxicity, but the temperature at which the insects are kept after treatment will have a large effect. In the first experiment a cool temperature of approximately 60° F. gave up to three times the average toxicity obtained at approximately 80° F., and in the second experiment the cool temperature gave up to five times the average toxicity obtained in the warm, and, taking only the experi-

TABLE 8. *Estimated relative potencies of pyrethrins to* Tribolium castaneum *Hbst., under various conditions*

Relative potency obtained by	Exp.	Mean*	Storage before spraying: Hot	Storage before spraying: Cold	Storage after spraying: Hot	Storage after spraying: Cold	Terpineol: Absent	Terpineol: Present
Cold instead of hot storage before spraying	I	1·39	—	—	1·43	1·35	1·19	1·62
	II	1·18	—	—	1·35	1·03	1·31	1·07
Cold instead of hot storage after spraying	I	3·16	3·25	3·08	—	—	2·25	4·45
	II	4·99	5·70	4·37	—	—	3·84	6·49
Addition of terpineol to spray	I	0·94	0·80	1·09	0·67	1·32	—	—
	II	1·37	1·51	1·24	1·05	1·78	—	—

* The means in this table have been obtained logarithmically and appear as geometric *not* arithmetic means.

In Table 8 the results of the two experiments are summarized as relative potencies. This table states, for example, that in the first experiment the mean effect of cold storage before spraying (averaged over the other two factors) was to increase the potency of the spray to 1·39 times its value for hot storage before spraying. The corresponding ratio for the four series of tests which were stored hot after spraying was 1·43, and for those stored cold *after* spraying was 1·35, those being averages over tests with and without terpineol. Again the ratio was 1·19 for the four series of tests without terpineol, 1·62 for the series with terpineol, these being averages for the alternative storage conditions after spraying. Other lines of the table are to be read in a similar manner. The lesser effect of cold storage before spraying in the second experiment is surprising since the exposure time was so much longer; it occurs only when the spray does not contain terpineol. In both experiments there is a marked effect of cold storage ments where terpineol was added to the spray, a maximum difference produced in both experiments was about seven times the toxicity under cool conditions as compared with hot.

These statements assume, as all the available evidence indicates, that the humidity differences occurring in the experiments were not an important factor.

EXP. III. 19 NOVEMBER 1942. THE EFFECT OF DIFFERENCES IN THE ENVIRONMENT BEFORE AND AFTER SPRAYING ON THE TOXICITY TO ADULT *TRIBOLIUM CASTANEUM* HBST., OF LAURYL THIOCYANATE IN AQUEOUS MEDIUM

Spraying conditions (see Table 1). Three replicates each of approximately twenty-five insects for each dilution.

Keeping conditions. H.H., H.C., C.H., C.C., as in Exps. I and II.

Procedure and general data

The procedure was the same as in Exps. I and II, except that the insects were put in the pre-cooled incubator at 56–58° F. 40 min. before spraying.

Solution: lauryl thiocyanate in 20 % v/v acetone, 0·1 % w/v sulphonated lorol.

General analysis of results

The lines derived from analysis are shown in Fig. 3. The results show that the toxicity of lauryl thiocyanate when the insects are put in cool storage is 1·47 times its toxicity when the insects are kept under the hot conditions.

TABLE 9. *Results of Exp. III*

Concentration lauryl thiocyanate	Mortality H.H.	Kill* (%)	Mortality H.C.	Kill* (%)	Mortality C.H.	Kill* (%)	Mortality C.C.	Kill* (%)
0·50	55/55	100	75/75	100	71/71	100	81/81	100
0·40	64/85	75	56/68	82	54/77	70	62/66	94
0·30	61/66	92	71/71	100	43/65	66	71/74	96
0·26	27/75	37	69/72	96	56/76	74	74/75	99
0·23	26/70	37	35/57	61	42/78	54	46/75	61
0·20	28/69	41	60/70	86	28/77	36	25/73	35
0·10	1/69	1	11/75	15	4/75	5	18/93	18
Sprayed control	0/69	—	0/65	—	0/78	—	1/73	—

* Corrected percentage allowing for mortality in the controls.

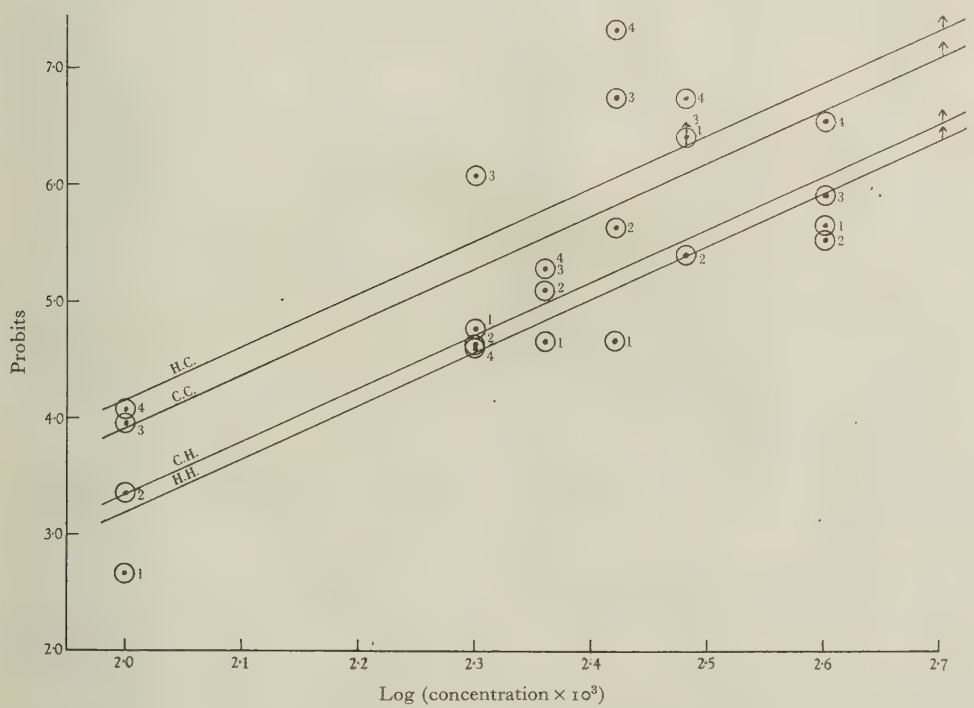

Fig. 3. Graph showing the effect of differences in the environment before and after spraying on the toxicity to adult *Tribolium castaneum* Hbst. of lauryl thiocyanate in aqueous medium. Graph shows the relation between probit mortality and log concentration after constraining the lines to be parallel. ↑ = dose at which 100% kill occurred.

1 = H.H. = Hot before treatment, hot after treatment
2 = C.H. = Cool before treatment, hot after treatment
3 = H.C. = Hot before treatment, cool after treatment
4 = C.C. = Cool before treatment, cool after treatment

Regression equations

H.H. $Y = -5{\cdot}96 + 4{\cdot}58x$
C.H. $Y = -5{\cdot}83 + 4{\cdot}58x$
H.C. $Y = -5{\cdot}02 + 4{\cdot}58x$
C.C. $Y = -5{\cdot}25 + 4{\cdot}58x$

Detailed analysis of results

The regressions of mortality probits on log concentration were all linear and did not depart significantly from parallelism, though there was marked heterogeneity in the behaviour of different batches of insects. The L.D. 50's as estimated from four parallel lines (Fig. 3) are set out in Table 10.

TABLE 10. L.D. 50 *of lauryl thiocyanate* (% *v/v*) *for adult* Tribolium castaneum *Hbst., under different conditions of treatment*

Before spraying	After spraying: Cold	Hot	Mean
Cold	0·172	0·231	0·199
Hot	0·153	0·247	0·195
Mean	0·163	0·239	0·197

incubator at 57–58° F., 30 min. before spraying commenced.

Solution: nicotine alkaloid in 20% v/v acetone, 0·1% w/v sulphonated lorol.

General analysis of results

The numerical results of probit analysis of these data are set out in the detailed analysis of results and the derived lines are shown in Fig. 4. There is a slight increase in the potency of the poison when cool conditions after treatment are contrasted with hot conditions after treatment, when the insects have been kept hot before treating.

Detailed analysis of results

Analysis of the data again showed that four parallel lines adequately fitted the probit mortality records, and again there was heterogeneity.

TABLE 11. *Results of Exp. IV*

Concentration nicotine (v/v %)	Mortality H.H.	Kill* (%)	Mortality H.C.	Kill* (%)	Mortality C.H.	Kill* (%)	Mortality C.C.	Kill* (%)
1·00	56/71	79	70/71	99	62/80	78	65/75	87
0·50	48/73	66	70/76	92	66/75	88	66/74	89
0·25	35/74	47	44/66	67	28/72	39	35/80	44
0·20	17/78	22	16/65	23	31/76	41	18/68	26
0·125	5/75	7	18/75	24	13/79	16	6/74	8
0·0625	2/62	3	2/82	2	4/79	5	4/83	5
Sprayed control	10/67	—	0/66	—	0/76	—	0/72	—

* Corrected percentage allowing for mortality in the controls.

The increase in toxicity due to cold storage after treatment is shown by the mean difference in the log L.D. 50, $-0{\cdot}166 \pm 0{\cdot}048$, an effect representing a potency for cold storage 147% of that for hot. The effect of storage temperature before spraying was negligible, there being a mean difference in L.D. 50 of $0{\cdot}101 \pm 0{\cdot}047$ corresponding to a cold storage potency 98% of that for hot storage. There is little evidence of any interaction between the two factors.

In order to extend these results a further test was done with nicotine.

EXP. IV. 26 NOVEMBER 1942. THE EFFECT OF DIFFERENCES IN THE ENVIRONMENT BEFORE AND AFTER SPRAYING ON THE TOXICITY TO ADULT *TRIBOLIUM CASTANEUM* HBST., OF NICOTINE IN AQUEOUS MEDIUM

Spraying conditions. See Table 1. Three replicates each of approximately twenty-five insects for each dilution.

Keeping conditions. H.H., H.C., C.H., C.C. as in Exps. I–III.

Procedure and general data

The procedure was the same as in Exps. I–III, except that the insects were put in the pre-cooled

The estimated values of the L.D. 50's are as indicated in Table 12.

TABLE 12. L.D. 50 *of nicotine* (% *v/v*) *for adult* Tribolium castaneum *Hbst. under different conditions of treatments*

Before spraying	After spraying: Cold	Hot	Mean
Cold	0·297	0·288	0·292
Hot	0·229	0·365	0·289
Mean	0·260	0·324	0·291

For this series the effect of cold storage after spraying is more complex. The average decrease in log L.D. 50 is $0{\cdot}094 \pm 0{\cdot}056$, corresponding to an increase in potency to 124% of the value for hot storage.

Closer examination of the table discloses that there was practically no difference in potency associated with storage temperature after spraying when the storage before spraying was cold; on the other hand, hot storage before spraying was followed by a 59% increase in potency for cold instead of hot storage after spraying. The average effect of cold storage before spraying was negligible, the difference in log L.D. 50 between hot and cold, $-0{\cdot}005 \pm 0{\cdot}056$,

representing a reduction of 1% in the potency for cold storage.

Without attempting to put forward any general explanation for the lack of differential effect found in this experiment, one condition of the experiment was thought to be worth further investigation. The practice of confining the insects on the sprayed surface by means of an inverted filter funnel is useful, because it is likely to promote even evaporation of the spray fluid from the sprayed surface and the sprayed insects, and thus to help in obtaining consistent results. However, it has the further effect of confining to some extent the vapour of the toxic substance, and while this is not likely to be an important effect with most of the materials used as contact poisons, it might be a major effect with nicotine, producing a marked fumigant action. It seems therefore that if an inverted filter funnel does produce a fumigant action, this action may be greater at the higher temperature than the lower, and will tend to neutralize any greater effect of the nicotine as a contact poison at the lower temperature.

To obtain further information an experiment using nicotine was carried out, in which the dishes were covered with muslin after treatment instead of an inverted filter funnel. The results of this experiment are set out below.

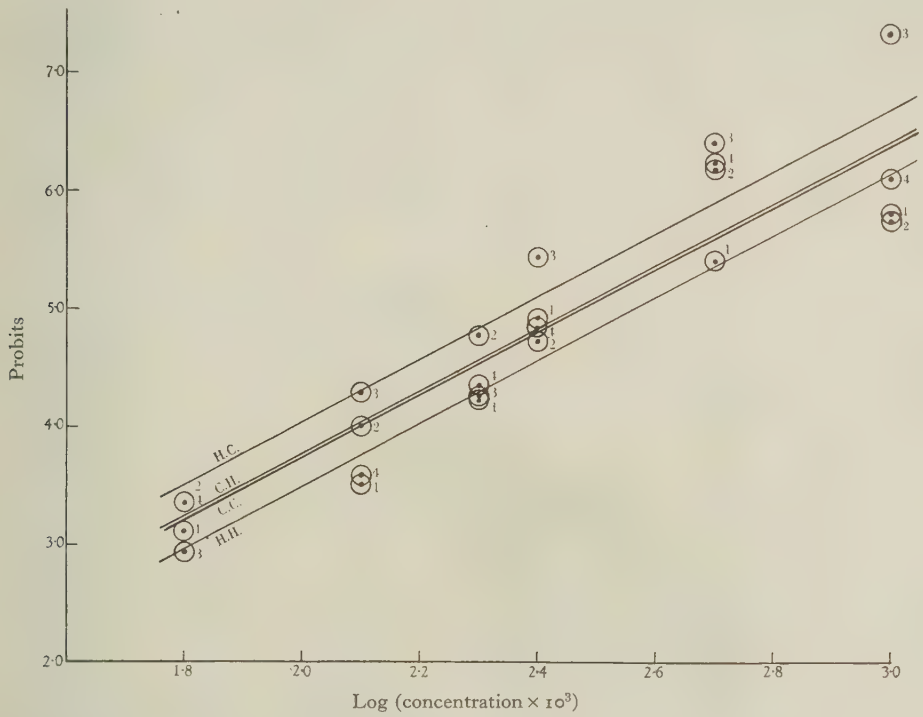

Fig. 4. Graph showing the effect of differences in the environment before and after spraying on the toxicity to adult *Tribolium castaneum* Hbst. of nicotine in aqueous medium. Graph shows the relation between probit mortality and log concentration after constraining the lines to be parallel.

	Regression equations
1 = H.H. = Hot before treatment, hot after treatment	H.H. $Y = -1{\cdot}78 + 2{\cdot}65x$
2 = C.H. = Cool before treatment, hot after treatment	C.H. $Y = -1{\cdot}51 + 2{\cdot}65x$
3 = H.C. = Hot before treatment, cool after treatment	H.C. $Y = -1{\cdot}25 + 2{\cdot}65x$
4 = C.C. = Cool before treatment, cool after treatment	C.C. $Y = -1{\cdot}55 + 2{\cdot}65x$

EXP. V. 3 DECEMBER 1942. EFFECT OF DIFFERENCES IN THE ENVIRONMENT BEFORE AND AFTER SPRAYING ON THE TOXICITY TO ADULT *TRIBOLIUM CASTANEUM* HBST., OF NICOTINE IN AQUEOUS MEDIUM (SECOND EXPERIMENT)

Spraying conditions. See Table 1. Five replicates each of approximately twenty-five insects used for each dilution.

Keeping conditions. H.H., C.C., these were as in Exps. I–IV, but the other treatments are omitted.

Procedure and general data

The procedure, except for the omission of the treatments H.C. and C.H. and the substitution of a muslin cover for the sprayed dishes instead of the inverted filter funnel, was the same as in Exps. I–IV; the insects were put in the pre-cooled incubator at 56–60° F. for 60 min. before spraying commenced. Temperatures and humidities are given in Table 1.

Solution: nicotine alkaloid in 20% v/v acetone; 0·1% w/v sulphonated lorol.

TABLE 13. *Results of Exp. V*

Concentration nicotine (v/v %)	Mortality H.H.	Kill* (%)	Mortality C.C.	Kill* (%)
1·6	25/135	16	104/137	69
0·8	9/131	5	28/126	15
0·4	5/125	2	13/122	4
0·3	5/116	3	12/124	3
0·2	2/133	—	20/129	9
0·15	0/129	—	10/118	2
Sprayed control	2/120	—	9/130	—

* Corrected percentage allowing for mortality in the control.

Detailed analysis of results

The figures do not merit exact statistical treatment.

General analysis of results

Although these figures cannot usefully be examined by probit analysis, particularly since only one concentration gave a kill of more than 20%, they are included because they strongly indicate two points. The first is the great importance of individual factors in procedure in toxicity experiments. Thus under very similar conditions the change from inverted filter funnels to muslin covers greatly decreased the toxicity of the nicotine under the conditions of storage in the hot room. This effect may be due to a lack of any fumigant effect when muslin covers were used, but it was observed that considerable condensation of the spray fluid occurred on the walls of the filter funnels when these were used, and it seems likely that the insects remained wet for much longer and therefore the contact effect also was likely to be increased.

In addition, the experiments strongly suggest that the hypothesis that the difference in toxicity obtained by different conditions of keeping would be influenced by the degree of confinement of the vapours of the spray solutions is correct. It appears that where little or no fumigant action can occur, cool storage after treatment increases toxicity.

It seems possible that the increased toxicity of poisons in the cool room, as opposed to the hot, found in the preceding experiments, might be due to the more rapid evaporation under the hot conditions of the spray fluid, particularly the water medium, or the more rapid volatilization or decomposition of the poison used. Further experiments were therefore carried out to test these points.

The data obtained with a solution of dinitro-*o*-cresol in ethylene glycol are set out in Exp. VI.

EXP. VI. 12 MARCH 1943. THE EFFECT OF DIFFERENCES IN THE ENVIRONMENT BEFORE AND AFTER SPRAYING ON THE TOXICITY TO ADULT *TRIBOLIUM CASTANEUM* HBST., OF A SOLUTION OF 3 : 5-DINITRO-*O*-CRESOL IN ETHYLENE GLYCOL

Spraying conditions. See Table 1. Two replicates each of approximately thirty insects for each dilution.

Keeping conditions. H.H., C.C. as in Exp. V.

Procedure and general data

The procedure was as in the previous experiments, inverted filter funnels being used to confine the insects. The insects were put in the pre-cooled incubator at 56° F. about 60 min. before spraying commenced.

Solution. 3 : 5-dinitro-*o*-cresol in ethylene glycol.

TABLE 14. *Results of Exp. VI*

Concentration dinitro-*o*-cresol (w/v %)	Mortality H.H.	Kill* (%)	Mortality C.C.	Kill* (%)
1·5	57/64	89	52/58	100
1·25	46/59	77	64/66	97
1·00	32/60	51	36/47	81
0·75	12/63	16	48/71	68
0·50	7/52	1	0/61	0
Sprayed control	2/56	—	0/65	—
Unsprayed control	3/64	—	0/51	—

* Corrected percentage allowing for mortality in the controls. Sprayed and unsprayed controls added together to give control figure.

An inspection of the sprayed dishes from the hot room 4 days after treatment showed them to be dry, i.e. the ethylene glycol had evaporated. The tricoline circles on which the insects were confined were stained yellow with dinitro-*o*-cresol and so were the inner surface of the filter funnels, indicating some volatilization of the dinitro-*o*-cresol.

The dishes from the cool room were not all dry: some of them had patches of liquid. Both the tricoline and the inner surface of the funnels were stained yellow with dinitro-*o*-cresol, but the wall of the funnels did not seem to be so heavily stained as those from the hot room.

In spite of the greater persistence of the ethylene glycol at the lower temperature of the cool room, it is to be noted that the controls were in better condition

than in the hot room, and that at the lowest concentration there was no kill.

General analysis of results

Analysis of the data and inspection of Fig. 5 show a marked increase in toxicity of the poison under the cool conditions.

Because the dinitro-*o*-cresol in ethylene glycol, although stable and relatively non-volatile, evaporated more rapidly from the dishes in the hot room than in the cool room, the factor of rapidity of evaporation cannot be ruled out as one of the important causes of the difference in toxicity under the two conditions. However, the experiment does indicate that humidity, influencing the evaporation of the water medium, used in the previous tests, is not the factor controlling the increase in toxicity under cool conditions, since the effect may be obtained with media, the evaporation of which is independent of the partial pressure of water vapour.

Detailed analysis of results

Analysis of the results showed that they were excellently fitted by two parallel lines relating the mortality probit to the log concentration; there was no heterogeneity between batches of insects. The derived lines are shown in Fig. 5. For cool storage the L.D. 50 is estimated to be 0·67 % w/v, and for hot storage 0·98 % w/v.

The difference between the logarithms of these L.D. 50's is $-0{\cdot}165 \pm 0{\cdot}020$, which is undoubtedly significant and corresponds to an increase in potency of 46 % for cool storage as compared with hot.

The next experiment was designed to test whether the differential effect of the two after-treatment conditions occurred when the insecticide was a highly refined white petroleum oil, a non-volatile, chemically inert substance. The results of this experiment are set out below.

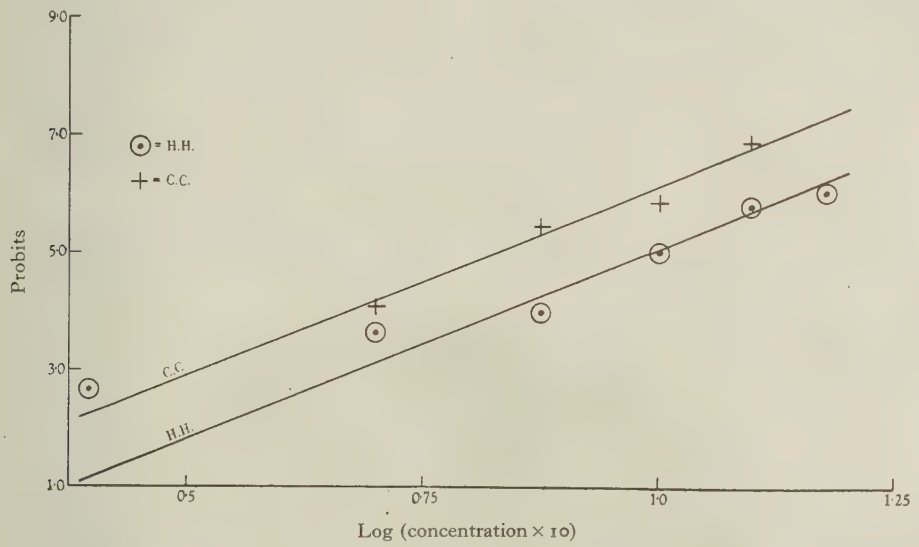

Fig. 5. Graph showing the effect of differences in the environment before and after spraying on the toxicity to adult *Tribolium castaneum* Hbst. of a solution of dinitro-*o*-cresol in ethylene glycol. Graph shows the relation between probit mortality and log concentration after constraining the lines to be parallel.

H.H. = Hot before treatment, hot after treatment
C.C. = Cool before treatment, cool after treatment

Regression equations
H.H. $Y = -1{\cdot}37 + 6{\cdot}44x$
C.C. $Y = -0{\cdot}31 + 6{\cdot}44x$

EXP. VII. 12 MAY 1943. THE EFFECT OF DIFFERENCES IN THE ENVIRONMENT BEFORE AND AFTER SPRAYING ON THE TOXICITY TO ADULT *TRIBOLIUM CASTANEUM* HBST., OF REFINED WHITE OIL

Spraying conditions. See Table 1. Three replicates of approximately 25 insects for each deposit level.

Keeping conditions. H.H. and C.C. as in Exps. V and VI.

Procedure and general data

The procedure was as in the previous experiments except that the insects were taken out of the cultures

TABLE 15. *Results of Exp. VII*

Fluid in reservoir (c.c.)	Mortality H.H.	Kill* (%)	Mortality C.C.	Kill* (%)
1	0/47	0	3/75	2
1·5	Rejected†	—	1/72	0
2·0	9/76	9	6/78	7
2·5	17/81	19	9/74	11
3·0	29/76	36	21/70	29
3·5	25/46	53	15/49	30
Sprayed control	2/75	—	—	—

* Corrected percentage allowing for mortality in the controls.

† Insects escaped in one of the replicates.

Note: A considerable number of the treated insects showed greatly swollen abdomens on inspection.

80% at 319–388° C.; flashpoint (closed) = 310° F., viscosity Redwood 1 at 70° F. = 104 (33·2 centistokes at 20° C.), sp. gr. at 15·5° C. = 0·880; unsulphonatable residue 88% by volume.

General analysis of results

The results of probit analysis of these data are given below, and these, together with the graph (Fig. 6), show that there was a greater toxicity in the hot room than in the cool room, for the first time in the series of experiments.

It is interesting to note that this result occurred with a substance that may be regarded as chemically inert, while the other results in the series were obtained using chemotoxic substances.

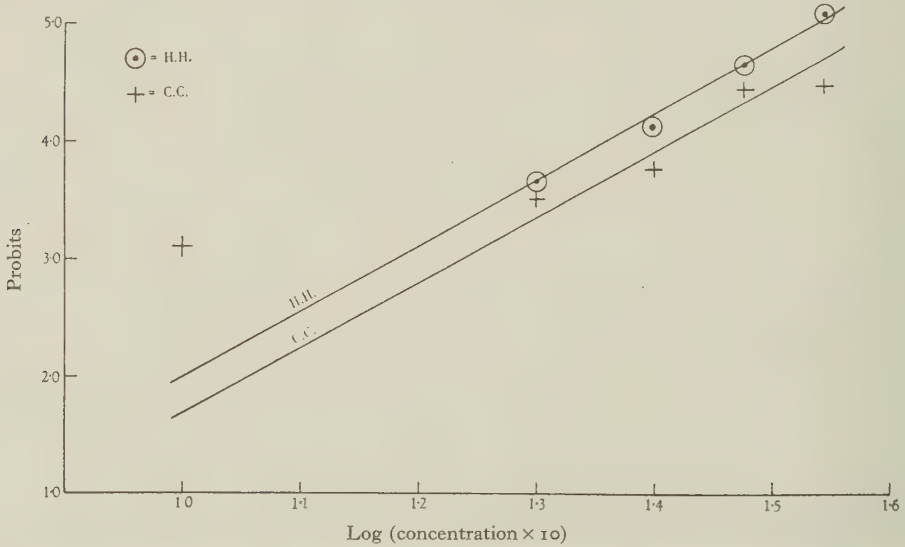

Fig. 6. Graph showing the effect of differences in the environment before and after spraying on the toxicity to adult *Tribolium castaneum* Hbst. of refined white oil. Graph shows the relation between probit mortality and log concentration after constraining the lines to be parallel.

H.H. = Hot before treatment, hot after treatment
C.C. = Cool before treatment, cool after treatment

Regression equation
H.H. $Y = -3{\cdot}40 + 5{\cdot}44x$
C.C. $Y = -3{\cdot}73 + 5{\cdot}44x$

the day before and left in the hot room overnight. Inverted filter funnels were used to confine the insects, but circles of grease-proof paper were placed in the dishes instead of tricoline, since the amount of oil necessary to kill the insects with a tricoline substratum would be excessive. The insects were put in the cool incubator at 59° F. 60 min. before spraying commenced.

Solution. Highly refined white oil* with the following specifications: 10% distilled at 298–319° C.,

* Wakefield half-white oil.

Detailed analysis of results

The mortalities in general were low, but two parallel probit regression lines were adequately fitted to the results and there was no heterogeneity. The derived lines are shown in Fig. 6. The estimated L.D. 50's are 4·03 c.c. for cool storage and 3·51 c.c. for hot. These figures are outside the range of concentration tested and consequently are not determined with any great precision.

Values for L.D. 10 are estimated at 2·34 and 3·04 c.c. The logarithmic difference between equally effective

concentrations is 0·060 ± 0·036. This significant value corresponds to a decrease of potency for cool storage to 87 % of the potency for hot storage.

In order to find out whether any difference in toxicity was shown under the hot and the cool conditions when both the poison and the medium were practically non-volatile, an experiment was made using a solution of D.D.T. (2:2-bis (*para*-chlorophenyl)-1:1:1-trichlorethane) in Wakefield half-white oil (see specification above). A thin layer of D.D.T. exposed in the hot room for more than a month showed no loss of weight.

The results of this experiment are set out below.

EXP. VIII. 27 JULY 1943. THE EFFECT OF DIFFERENCES IN THE ENVIRONMENT BEFORE AND AFTER SPRAYING ON THE TOXICITY TO ADULT *TRIBOLIUM CASTANEUM* HBST., OF A SOLUTION OF D.D.T. (2:2-BIS (*PARA*CHLOROPHENYL)-1:1:1-TRICHLORETHANE) IN REFINED WHITE OIL

Spraying conditions. See Table 1. One replicate of approximately twenty-five insects for each deposit level for each condition of treatment.

Keeping conditions. H.H. and C.C. as in Exps. V, VI and VII.

Procedure and general data

Procedure was as in the preceding experiments, except that the insects were put into tubes on the afternoon of treatment. They were placed in the cool incubator at 51–55° F. approximately 70 min. before treatment commenced. The dishes had a tricoline circle in the bottom and were covered with an inverted filter funnel after treatment. Temperatures are given in Table 1.

TABLE 16. *Results of Exp. VIII*

Concentration D.D.T. (w/v %)	Mortality H.H.	Kill* (%)	Mortality C.C.	Kill* (%)
5·0	25/25	100	16/16	100
3·6	23/23	100	15/15	100
3·125	23/23	100	24/24	100
2·5	Insects escaped	—	23/23	100
1·875	21/25	82	23/23	100
1·25	20/22	90	25/25	100
0·625	3/20	7	19/25	76
0·3125	3/23	0	14/29	48
Control W.H.W.O.	4/29	—	0/18	—
Control unsprayed	1/24	—	0/23	—

* Corrected percentage allowing for mortality in the controls. The sprayed and unsprayed controls were added together to give the control figure.

The insects were inspected 31 July, 5 Aug. and 9 Aug. 1943. The figures for the inspection of 5 Aug. were taken since these were clear cut, and by 9 Aug. there was a fair mortality in the controls. It was noteworthy that the insects took longer to die at the lower temperature.

General analysis of results

From the analysis given below it will be seen that once again a greater toxic effect is produced with the poison when the insects are kept under the cool storage conditions after treatment than when they are kept under warm conditions. The poison is 2·61 times as toxic under the cool conditions than it is under the hot. This is a greater increase than that found with any of the other poisons, except the pyrethrins.

This experiment indicates that with some poisons the increased recovery at the higher temperatures is not due to the more rapid volatilization of the poison or to the more rapid evaporation of the solvent medium.

Detailed analysis of results

The results were adequately fitted by two parallel probit regression lines and there was no heterogeneity of departure from them. The lines are shown in Fig. 7.

The estimated L.D. 50's are 0·95 % w/v for hot storage and 0·36 % w/v for cold; the logarithmic difference of $-0{\cdot}417 \pm 0{\cdot}056$ is a clearly significant value, and represents a potency for cold storage 2·61 times that for hot.

DISCUSSION

The early experiments showed that with the conditions chosen, the greatest difference in effect was produced by the storage after treatment, the conditions prior to treatment having much less effect. For the later experiments, therefore, a standard procedure was adopted before treatment, and the effect of the different after-treatments noted. Table 17 summarizes the data on changes in toxicity due to different treatment before and after spraying.

It seems probable that the increase in toxicity under the cool conditions of after-treatment is due to the physiological conditions of the insect at these temperatures, and not to the effect of these temperatures on the poison or the medium in which it is carried. The evidence for this statement is that the increase occurred when chemically stable and chemically unstable, when volatile and non-volatile, when liquid and solid poisons and when volatile or non-volatile media were used. Since the cooling before treatment was not as prolonged as the cooling after treatment, it would need further study before the two effects could be satisfactorily compared. However, in the one instance, that of the pyrethrins, where the effect of cooling before treatment for 1 hr. was compared with the effect for 24 hr., the extended period for cooling had little effect, and it was the

reverse of the expected, i.e. tended toward a decrease in toxicity.

It seems likely, therefore, that, within the limits set by the experiments, cooling before treatment has much less effect on subsequent toxicity than cooling after treatment.

It must be emphasized that the results detailed in this paper were obtained using a single insect species and that species specialized to withstand indoor conditions. Some preliminary experiments on plant-feeding insects which we hope to amplify and publish later have not so far given such clear-cut results. This may be due to the greater sensitivity of the plant-feeding species to the different conditions, the difference in environment alone producing an effect overriding that of the poison. In addition, owing to lack of adequate facilities, the humidity data are incomplete and unsatisfactory. The conclusions that

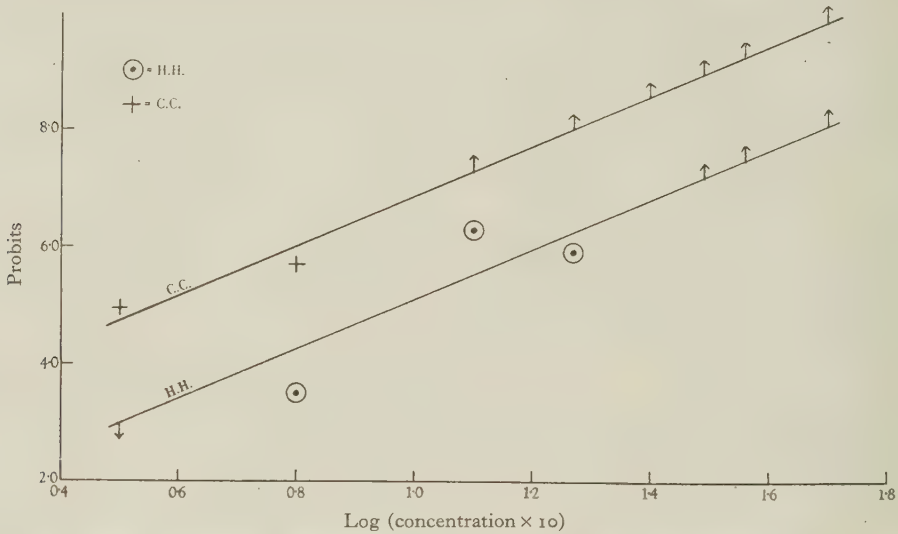

Fig. 7. Graph showing the effect of differences in the environment before and after spraying on the toxicity to adult *Tribolium castaneum* Hbst. of a solution of D.D.T. (2:2-bis (*para*chlorophenyl)-1:1:1-trichlorethane) in Wakefield half-white oil. Graph shows the relation between probit mortality and log concentration after constraining the lines to be parallel. ↑ = dose at which 100% kill occured. ↓ = dose at which 0% kill occurred.

	Regression equations
H.H. = Hot before treatment, hot after treatment	H.H. $Y = 0{\cdot}88 + 4{\cdot}22x$
C.C. = Cool before treatment, cool after treatment	C.C. $Y = 2{\cdot}64 + 4{\cdot}22x$

TABLE 17. *The effect of cool storage compared with hot, on the toxicity of various contact poisons to adult* Tribolium castaneum *Hbst.*

		Potency under cold storage as proportion of that under hot		
Poison	Medium	Cold before spraying	Cold after spraying	Cold before and after spraying
Pyrethrins*	Aqueous	1·19, 1·31	2·25, 3·84	2·67, 5·01
Pyrethrins and terpineol*	Aqueous	1·62, 1·07	4·45, 6·49	7·21, 6·90
Lauryl thiocyanate	Aqueous	0·98	1·47	1·43
Nicotine	Aqueous	0·99	1·24	1·23
Dinitro-*o*-cresol	Ethylene glycol	—	—	1·46
D.D.T.	Wakefield oil	—	—	2·61
Wakefield half-white oil	—	—	—	0·87

* Two experiments.

have been drawn must therefore be regarded as limited in significance and in need of confirmation, but there seems to be little doubt of the main conclusions that the atmospheric environments before and after treatment, particularly after treatment, produce a difference which may be large in the toxicity of contact poisons, and that the degree of this difference varies with the poison used.

Considering only the problem of laboratory estimation of toxicity these results indicate that an alteration of the environment, particularly the environment after treatment may affect, not only the absolute but also the relative toxicity of the poisons studied.

We have to acknowledge the constant help of Mr D. J. Finney of the Statistical Department of this station. He suggested the statistical methods employed and was responsible for a considerable number of the calculations. He also provided the statistical data and analysis for the sections on the detailed analysis of results.

We are indebted also to Dr Tattersfield for help and criticism through the course of this work.

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The analysis of a factorial series of insecticide tests

By D. J. Finney, *Rothamsted Experimental Station, Harpenden, Herts.*

When a set of insecticidal toxicity tests yields parallel regression lines for the relationship between mortality probit and log dose, the potencies of the materials or conditions under test may be compared purely in terms of log L.D. 50's. The purpose of this paper is to suggest that, when tests have been made with all combinations of several different factors, standard methods for the statistical analysis of factorial experiments may be adapted to the examination of the relative potencies.

Data obtained by Potter & Gillham (1946), in a 2^3 factorial experiment on alternative storage conditions for insects before and after spraying and the adjuvant action of terpineol in a pyrethrins spray, are used in an example of the computations. Details are given of the test of parallelism of the regression lines, the factorial analysis of the log L.D. 50's, the estimation of the mean effects and interactions and their standard errors, the significance tests, and the preparation of summary tables.

Introduction

The desirability of planning insecticide tests so as to investigate, in one experiment, several different factors defining the dose and conditions of testing has been emphasized elsewhere (Finney, 1942); this is indeed only one aspect of the usefulness of factorial design in biological investigations (Fisher, 1942). The method of statistical analysis appropriate to experiments in which two or more quantitative factors have been employed has already been discussed (Finney, 1943), but certain points of interest of a rather different nature arise when some factors are qualitative. The later stages of the analysis can then be put into a form which is essentially the same as that in common use for analogous factorial experiments on agricultural problems, though a complication is introduced by the unequal variances of the quantities compared.

Potter & Gillham (1946) have reported the results of several insecticide experiments of simple factorial design, and one of these gives a useful example of the computations required for a full statistical analysis. The account which follows does not pretend to be an exhaustive study of the general problem, though the method of analysis can be adapted to suit other factorial arrangements.

Design of the experiment

Potter & Gillham carried out two experiments on the toxicity of pyrethrins to adult *Tribolium castaneum* Hbst. using various conditions of storage before and after spraying. Full details are given in their paper (1946); for the present purpose all that need be noted is that the insects were stored in either a hot (H.) or a cold (C.) room before spraying, and also in either a hot or a cold room after spraying. Tests were made with each of the four possible combinations of storage treatments. Tests were also made, with each of these conditions, using a spray to which terpineol (T.) had been added. The eight series of tests form what is called a $2 \times 2 \times 2$ or 2^3 factorial system, there being three factors (storage of insects before spraying, storage after spraying, terpineol), each of which has two alternative states (hot or cold for the first two, absence or presence for the third).

The results of Potter & Gillham's first experiment, which will be used here in an example of the statistical analysis, are given in Table 1. The eight series are identified by code symbols, C.H.T. representing the tests in which terpineol was added to the spray for insects stored cold before, hot after, spraying. For this experiment, in each series about seventy insects were tested at each of three doses, 0·0195, 0·0130 and 0·0065 % w/v total pyrethrins; Table 1 shows the number killed as a fraction of the number tested at each dose, and this quantity expressed as a percentage. Control batches indicated a death-rate of about 1 % amongst unsprayed insects, irrespective of storage treatment.

Estimation of median lethal dosages

The first stage in the statistical reduction of the results is the estimation of the median lethal dosages. Here, as is usual in insecticide tests, the dosage is measured by the log concentration of pyrethrins, to which the probit of the mortality is linearly related; in order to avoid negative quantities, all concentrations were multiplied by 1000 before logarithms were taken. Standard methods of probit analysis (Bliss, 1935*a*, *b*; Fisher & Yates, 1943) need little modification for use in factorial experiments, and only a brief outline of this stage need be given. Allowance for the natural mortality rate of 1 % has been made by taking the appropriate weighting coefficients from Finney's (1944) table and adopting the approximate

method of analysis which he has suggested for use when the control mortality is small.

The probits of the percentage mortalities (adjusted for controls) at each concentration in each series were plotted against the logarithm of 1000 × concentration. The resulting diagram (Potter & Gillham's Fig. 1) suggested that the eight regression lines of mortality probit on log concentration of pyrethrins had very similar slopes; the provisional lines were therefore all drawn parallel and used to initiate the usual computations for fitting improved estimates of each. The mean dosage ($\bar{x}$), the mean values of S_{xy} and S_{xx} for each series separately depart significantly from the average, b. Bliss (1935b) indicated how the test of parallelism should be made, and Cochran (1938) gave an example in which he used a formula applicable only to a pair of lines. When there are more than two lines a comprehensive test of all at once is preferable to the separate examination of every pair, and the form used below is a convenient computational adaptation of Bliss's equation (20a). The quantity S_{xy}^2/S_{xx} is calculated for each series, and entered in Table 2; it represents the portion of S_{yy} accounted for by a regression line

TABLE 1. *Results of toxicity tests on* Tribolium castaneum *Hbst. (Potter & Gillham,* 1946)

Total pyrethrins (% w/v)	H.H.		C.H.		H.C.		C.C.	
	Mortality	% kill	Mortality	% kill	Mortality	% kill	Mortality	% kill
0·0195	39/44	89	63/70	90	75/75	100	80/80	100
0·0130	28/50	56	56/72	78	65/72	90	79/80	99
0·0065	8/60	13	16/70	23	48/63	76	61/85	72
Control	1/41	2	1/60	2	1/70	1	0/73	0
Total pyrethrins (% w/v)	H.H.T.		C.H.T.		H.C.T.		C.C.T.	
	Mortality	% kill	Mortality	% kill	Mortality	% kill	Mortality	% kill
0·0195	37/63	59	60/76	79	70/70	100	74/74	100
0·0130	10/76	13	34/75	45	45/49	92	69/70	99
0·0065	3/68	4	16/71	23	47/60	78	65/69	94
Control	0/68	0	1/75	1	1/72	1	2/58	3

Percentage kills in the table have not been adjusted for the control mortality of 1%.

TABLE 2. *Summary of calculations for fitting probit lines*

Series	$\bar{x}$	$\bar{y}$	Snw	S_{xx}	S_{xy}	S_{yy}	S_{xy}^2/S_{xx}
H.H.	1·0572	4·9656	76·0	2·7375	13·179	63·98	63·45
C.H.	1·0590	5·3702	103·9	3·7137	16·478	74·63	73·11
H.C.	0·9526	6·0550	63·3	2·0083	6·075	21·92	18·38
C.C.	0·9081	6·0737	63·6	1·6054	7·974	39·71	39·61
H.H.T.	1·1484	4·4072	92·7	2·2607	10·174	52·30	45·79
C.H.T.	1·0885	5·0031	115·3	3·9490	12·457	43·17	39·30
H.C.T.	0·9175	6·0617	47·4	1·3523	4·309	16·23	13·73
C.C.T.	0·8640	6·6923	24·2	0·3735	1·117	3·58	3·34
Total	—	—	—	18·0004	71·763	315·52	296·71

$S_{xx} = Snw\,(x-\bar{x})^2$, $S_{xy} = Snw\,(x-\bar{x})\,(y-\bar{y})$, $S_{yy} = Snw\,(y-\bar{y})^2$.

probit ($\bar{y}$), and the sums of squares and products for the eight series are shown in Table 2.

This table provides the material for calculating the equations for a series of eight parallel lines fitted to the data. The common slope, b, of these lines is taken from the totals in the table:

$$b = \Sigma S_{xy}/\Sigma S_{xx}$$
$$= 71{\cdot}763/18{\cdot}0004$$
$$= 3{\cdot}9867.$$

Before making use of this slope in the estimation of the log L.D. 50 for each series, a test should be made of whether the hypothesis of parallelism of the eight lines accords with the data, or, in other words, of whether regression coefficients calculated from the calculated for that series alone. The total of this column is the portion of ΣS_{yy} (= 315·52) accounted for by the fitting of eight lines not constrained to be parallel. From the total line of Table 2 is calculated

$$(\Sigma S_{xy})^2/\Sigma S_{xx} = (71{\cdot}763)^2/18{\cdot}0004$$
$$= 286{\cdot}10;$$

this is the portion of ΣS_{yy} accounted for by fitting eight parallel lines, one for each series. The first, third, and fifth lines of the analysis of variance shown in Table 3 have now been calculated, and the remaining two can be obtained by subtractions. The fourth line is in fact the sum of the heterogeneity χ^2 values for each test, each with 1 degree of freedom, obtained as $\chi^2 = S_{yy} - S_{xy}^2/S_{xx}$;

this must be tested for significance as a χ^2 with 8 degrees of freedom. Fisher & Yates (1943, Table IV) give 15·51 as the 5 % significance level, and the heterogeneity of the mortalities about the values predicted by the eight separate regression lines must therefore be judged significant. The significance of the component representing departures from parallelism must now be assessed by comparison of the mean squares in the last column of Table 3, using Fisher & Yates's Table V (1943); the 5 % level for the ratio is 3·50, whereas the value in Table 3 is less than 1 and therefore not significant.* Hence in the present example relative potencies may be determined by using the average regression coefficient, b, to give the probit regression lines for every series.

TABLE 3. *Analysis of variance of mortality probits*

	Degrees of freedom	Sum of squares	Mean square
Common slope	1	286·10	—
Departures from parallelism	7	10·61	1·52
Separate slopes	8	296·71	—
Heterogeneity	8	18·81	2·35
Total	16	315·52	

From the values of $\bar{x}$ and $\bar{y}$ given in Table 2, the eight parallel probit regression equations can be computed; the first, for example, is

$$Y = 4{\cdot}9656 + 3{\cdot}9867\,(x - 1{\cdot}0572)$$
$$= 0{\cdot}751 + 3{\cdot}987x.$$

The estimated log L.D. 50 for any poison is then that value of x which gives $Y = 5$ in the appropriate equation; these estimates are shown in Table 4, in the column headed m. The variance of any m is

$$V(m) = \frac{1{\cdot}96}{b^2}\left\{\frac{1}{Snw} + \frac{(m-\bar{x})^2}{\Sigma S_{xx}}\right\},$$

where Snw is the sum of weights for the particular series and ΣS_{xx} is the total of S_{xx} over all series. (Had there been no heterogeneity, the factor 1·96 would be omitted from this expression for the variance.)

Comparison of potencies

Inspection of the values of m shows that, other things being equal, the insects stored cold after spraying had a much lower log L.D. 50 than those stored hot. Cold storage before spraying, as compared with hot, had a similar but much less marked effect. Terpineol seemed to affect the log L.D. 50 in a somewhat irregular manner. A very convenient method of studying the effects and their interactions in an experiment of this design (a 2^n design, using several factors each at two levels) has been suggested by Yates (1937, §3). A full account of the method will not be given here, but the computations are shown in Table 4; the reader who is unfamiliar with the analysis of factorial experiments should first read the relevant sections of Yates's paper if he wishes to understand fully the technique employed.

TABLE 4. *Values of log* L.D. 50 *and calculation of treatment effects*

Series	m	(1)	(2)	(3)	Means	(3) for $\bar{x}$
H.H.	1·066	2·032	3·359	6·834	0·854	7·995
C.H.	0·966	1·327	3·475	−0·570	−0·142 ± 0·034	−0·156
H.C.	0·688	2·385	−0·149	−2·000	−0·500 ± 0·042	−0·711
C.C.	0·639	1·090	−0·421	0·048	0·012 ± 0·032	−0·040
H.H.T.	1·297	−0·100	−0·705	0·116	0·029 ± 0·032	0·042
C.H.T.	1·088	−0·049	−1·295	−0·272	−0·068 ± 0·033	−0·071
H.C.T.	0·651	−0·209	0·051	−0·590	−0·148 ± 0·033	−0·200
C.C.T.	0·439	−0·212	−0·003	−0·054	−0·014 ± 0·032	0·053

In order to allow for the heterogeneity, all variances should be increased by a heterogeneity factor, which strictly is the mean square 2·35 shown in Table 3, and the variances would then have 8 degrees of freedom; here it seems reasonable to obtain a more precise estimate of the factor by combining the sums of squares for 'heterogeneity' and 'parallelism' to give a mean square of

$$(10{\cdot}61 + 18{\cdot}81)/(7+8) = 1{\cdot}96$$

with 15 degrees of freedom. Hence, for example, the variance of the regression coefficient is

$$V(b) = 1{\cdot}96/18{\cdot}0004$$
$$= 0{\cdot}1089.$$

The estimates of log L.D. 50 are arranged in a systematic order, starting with 'hot before and after, no terpineol' and changing each factor in turn in the manner shown. Column (1) is then formed from column m. The first four entries are the sums of the four successive pairs of values of m and the last four entries are the differences of these pairs, the first always being subtracted from the second. Thus

$$0{\cdot}966 + 1{\cdot}066 = 2{\cdot}032, \quad 0{\cdot}966 - 1{\cdot}066 = -0{\cdot}100.$$

* Had there been no heterogeneity, the sum of squares for 'parallelism' would have been tested as a $\chi^2_{[7]}$. When only two lines are being compared, the method of calculation used here gives the same value to the $\chi^2_{[1]}$ for parallelism as in Cochran's example; the method is no more laborious than his, and has the advantage of easy generalization to a greater number of lines.

Column (2) is derived from column (1) and column (3) from column (2) by the same process.

The first entry in column (3) is then the total of the eight values of m, and division by eight gives the general mean shown in the next column. The other entries in column (3) are divided by four and the result is entered in the next column. The second entry in the column of means is easily verified to be the mean difference between the four values of m for 'cold before spraying' and the four for 'hot before spraying'. The third entry is the corresponding difference for storage after spraying. The fourth entry is one-half the amount by which the difference in m for alternative after-spraying conditions in the four series stored cold before spraying exceeds the corresponding difference in the four series stored hot before spraying, and is defined to be the measure of the *interaction* between the two storage treatments. The mean difference between hot and cold after spraying in the four series stored cold before spraying is

$$\tfrac{1}{2}(0{\cdot}639+0{\cdot}439)-\tfrac{1}{2}(0{\cdot}966+1{\cdot}088)=-0{\cdot}488,$$

and the corresponding difference in the series stored hot before spraying is

$$\tfrac{1}{2}(0{\cdot}688+0{\cdot}651)-\tfrac{1}{2}(1{\cdot}066+1{\cdot}297)=-0{\cdot}512.$$

One-half the sum of these is the mean effect of cold after spraying, $-0{\cdot}500$, and one-half their difference is the measure of interaction, 0·012.

All these four entries are averages for series with and without terpineol. The fifth entry is the mean effect of terpineol on m, or the average difference between the four tests with terpineol and the four without. The sixth and seventh entries are the interactions of the terpineol effect with storage condition before spraying and storage condition after spraying respectively, and the last entry is a measure of the interaction between all three factors. These interactions are defined in a manner similar to that discussed above. Fuller description and explanation cannot be given here, but will be found in Yates's monograph, which though written primarily from the point of view of agricultural experimentation, has many applications in the biological laboratory.

The variance of any one of the main effects or interactions has an expression analogous to $V(m)$, and is in fact

$$V=\frac{1{\cdot}96}{16b^2}\left\{\Sigma\left(\frac{1}{Snw}\right)+\frac{\delta^2}{\Sigma S_{xx}}\right\};$$

the first term within the brackets is the sum of the reciprocals of the eight Snw and, in the second term, δ is the difference between the appropriate entry in column (3) of Table 4 and the corresponding quantity formed from $\bar{x}$ instead of from m. These totals for $\bar{x}$ are listed in the last column of Table 4, but details of their computation are not shown as the method is the same as that used to give column (3). The factor of 16 in the denominator arises as the square of four, the contrasts examined being differences between means of two groups of four series, and allowance for heterogeneity is again made by means of the factor 1·96. For example, for the effect of storage conditions after spraying

$$\delta=-2{\cdot}000+0{\cdot}711=-1{\cdot}289,$$

and therefore the variance of the effect is

$$V(M)=\frac{0{\cdot}122}{b^2}\left\{0{\cdot}1362+\frac{(1{\cdot}289)^2}{18{\cdot}0004}\right\}$$

$$=(0{\cdot}042)^2.$$

The standard errors shown in Table 4 are the square roots of these variances.

Summarizing the results

Comparison of the mean effects and interactions with their standard errors indicates that storage conditions, either before or after spraying, have significantly affected the potency of the spray, but that their interaction is small and well within the limits of error.* The over-all terpineol effect is not significant, but the magnitude of its interactions with the storage treatments suggests that it should be examined in more detail.

In Table 5 the influence of storage conditions after spraying on the log L.D. 50 is shown separately for the series without and with terpineol; the table has been calculated by averaging values of m from pairs of series which differ only in storage conditions before spraying. The last line of this table shows the mean effect of terpineol to have been an increase of 0·029 in the log L.D. 50 (as already given in Table 4), a non-significant amount. But in the series stored hot after spraying, the difference is $0{\cdot}176\pm0{\cdot}037$, and in the series stored cold the log L.D. 50 is *decreased* by $0{\cdot}119\pm0{\cdot}054$; both these differences are significant, the first representing a 33% decrease in potency when terpineol is added to the pyrethrins and the second a 32% increase in potency.

The variances of the differences in log L.D. 50 have been obtained from the formula

$$V(M)=\frac{1{\cdot}96}{4b^2}\left\{\Sigma'\left(\frac{1}{Snw}\right)+\frac{\delta^2}{18{\cdot}0004}\right\}.$$

Here the factor 4 in the denominator is the square of 2, since the contrast is a difference between means of two series; Σ' represents summation over the four series (either the four hot after spraying or the four

* $t^2V(b)/b^2=0{\cdot}031$, a value small enough for tests of significance and fiducial limits based on standard errors of relative potencies to be sufficiently accurate (Cochran, 1938).

TABLE 5. *The effect of storage conditions after spraying and of terpineol on the mean values of the log* L.D. 50

(Results average over alternative storage conditions before spraying.)

After spraying	Addition of terpineol to spray: Absent	Present	Mean
Hot	1·016	1·192	1·104
Cold	0·664	0·545	0·604
Mean	0·840	0·869	0·854

TABLE 6. *The effect of storage conditions after spraying and of terpineol on the mean values of the* L.D. 50

(Anti-logarithms of Table 5; % w/v total pyrethrins.)

After spraying	Addition of terpineol to spray: Absent	Present	Mean
Hot	0·0104	0·0156	0·0127
Cold	0·0046	0·0035	0·0040
Mean	0·0069	0·0074	0·0071

Table 6. This table is the same as the first part of Potter & Gillham's Table 7; the corresponding parts of their Tables 5 and 6 can be obtained in the same manner from other pairs of factors, averaging out either conditions after spraying or the terpineol treatment. The contrast in the terpineol effects for different storage conditions before spraying is less marked, but again there is an indication that terpineol reduces the potency in the hot series, increases it in the cold.

A simple and convenient form of summary of relative potencies in experiments of this type is illustrated in Table 8. This may be calculated directly from Table 6 and the two analogous tables not given here, or perhaps more easily by way of Table 7. The latter table shows differences between equally effective doses, firstly for the over-all effects of single factors and secondly for the interactions of two factors. The 'mean' column is taken from suitable entries in the 'mean' column of Table 4 (with a reversal of sign) and states that, for example, the average effect of cold storage before spraying is to

TABLE 7. *Estimated relative dosage values (measured on logarithmic scale)*

Relative dosage value for	Mean	Storage before spraying: Hot	Cold	Storage after spraying: Hot	Cold	Terpineol: Absent	Present
Cold instead of hot storage before spraying	0·142	—	—	0·154	0·130	0·074	0·210
Cold instead of hot storage after spraying	0·500	0·512	0·488	—	—	0·352	0·648
Addition of terpineol to spray	−0·029	−0·097	0·039	−0·177	0·119	—	—

cold) and δ is the difference between the two expressions of the form

$$(m_1+m_2-m_3-m_4) \quad \text{and} \quad (\bar{x}_1+\bar{x}_2-\bar{x}_3-\bar{x}_4).$$

For example, the effect of terpineol on the log L.D. 50 in the series stored hot after spraying is obtained from

$$1{\cdot}297+1{\cdot}088-1{\cdot}066-0{\cdot}966=0{\cdot}353,$$

one-half of which is the value 0·176 previously quoted. The corresponding difference in values of $\bar{x}$ is, from Table 3,

$$1{\cdot}1484+1{\cdot}0885-1{\cdot}0572-1{\cdot}0590=0{\cdot}121.$$

Hence the variance of the mean difference 0·176 is

$$V(M)=\frac{0{\cdot}49}{b^2}\left\{0{\cdot}0423+\frac{(0{\cdot}232)^2}{18{\cdot}0004}\right\}$$
$$=(0{\cdot}037)^2.$$

The anti-logarithms of the entries in Table 5, divided by 10^3, are the estimated L.D. 50's (on the concentration scale) for the different treatment combinations, and the table thus derived is shown as

make any dosage (or log concentration) as effective as a dosage 0·142 greater with hot storage. The corresponding separate values for the series stored hot or cold after spraying are obtained respectively by subtracting or adding the interaction value, 0·012, before the reversal of sign:

$$-0{\cdot}142-0{\cdot}012=-0{\cdot}154,$$
$$-0{\cdot}142+0{\cdot}012=-0{\cdot}130.$$

Similarly, subtraction and addition of the interaction −0·068 gives the values without and with terpineol respectively.

Table 8 is then constructed by taking anti-logarithms of the entries in Table 7; the same values may be obtained as the ratios of the appropriate L.D. 50's in Table 6 and analogous tables. The table states that the average effect of cold storage before spraying (as compared with hot) was to increase the potency by 39%. For the series stored hot after spraying this increase was 43% and for the series stored cold after spraying it was 35%; for the four series without terpineol the increased potency was 19% and for the four series with terpineol 62%. The

TABLE 8. *Estimated relative potencies*

Relative potency for	Mean	Storage before spraying		Storage after spraying		Terpineol	
		Hot	Cold	Hot	Cold	Absent	Present
Cold instead of hot storage before spraying	1·39	—	—	1·43	1·35	1·19	1·62
Cold instead of hot storage after spraying	3·16	3·25	3·08	—	—	2·25	4·45
Addition of terpineol to spray	0·94	0·80	1·09	0·67	1·32	—	—

second line gives similar information on the effect of storage conditions after spraying as expressed by the potency of cold relative to hot. The third line gives the average potency of the terpineol series relative to those without terpineol and also values of this relative potency under different storage conditions.

I am indebted to Dr C. Potter and Mrs E. M. Gillham for advice in the preparation of this paper and for permitting me to use their data in an example of the computational procedure.

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Note on the effect of wireworms of the genera *Agriotes* and *Corymbites* on crop yields

By L. Broadbent, B.Sc., *Midland Agricultural College, Sutton Bonington, Loughborough*

(With 2 Text-figures)

Experiments were made to illustrate the regular falling off of yield of crops due to increasing wireworm populations under controlled conditions. The genus *Corymbites* was shown to cause as much damage as *Agriotes*; *Athous* was less harmful. The damage caused to various cereals by *Agriotes* is contrasted.

General observations

During 1941, 1942 and 1943 all crops grown on fields sampled, while still under old turf, by the wireworm survey teams in the Midland Province were assessed as satisfactory, poor or failure from returns supplied by the farmers and from inspections of the growing crops. It soon became apparent that the sampling and hand-sorting methods used were affording results upon which advice regarding cropping could be given, and it became possible to assess the chances of a crop against an estimated wireworm population. The populations were obtained by counting only those wireworms over 5 mm. long. Salt & Hollick (1944) showed that the population thus estimated is about one-third of the actual total, but it includes all the larvae likely to damage crops.

Table 1 gives the percentage of satisfactory crops grown on fields with low (0–300,000 wireworms per acre), medium (300,000–600,000), high (600,000–1,000,000) or very high (over 1,000,000) populations. The figures are based on the final crop results and therefore include all factors adversely affecting the crops. Thus in the case of beans the percentages are much lower than if the effect of wireworms only had been taken into account, since the crop suffered severely from attacks of *Aphis* (*Doralis*) *fabae* Scop. in the years under consideration.

Some of these figures are incorporated in the report on the national survey of wireworms (1944). The statistical aspect of such tables has been discussed by Finney (1941).

Table 1. *Percentage of satisfactory crops. The number of fields examined is given in brackets*

	Wireworm population			
	Low	Medium	High	Very high
Wheat (mainly winter)	91 (89)	76 (112)	68 (95)	46 (46)
Oats (mainly spring)	91 (182)	79 (188)	62 (120)	41 (74)
Oats and pulse	100 (30)	75 (24)	66 (15)	78 (9)
Rye	80 (10)	83 (6)	66 (3)	0 (3)
Barley	75 (16)	90 (30)	59 (29)	60 (25)
Peas	89 (18)	77 (44)	71 (52)	68 (66)
Beans	86 (7)	80 (20)	45 (31)	79 (29)
Roots and greens	89 (54)	70 (60)	69 (51)	46 (39)
Potatoes	91 (57)	74 (50)	50 (18)	55 (9)
Flax	95 (21)	92 (47)	95 (37)	93 (58)
Seeds	100 (31)	82 (22)	89 (19)	70 (10)
Average % and total yield	91 (515)	78 (603)	67 (470)	62 (368)

It was noted during the early years of the survey that the genus *Corymbites*, which occurred in small numbers in lowland fields, largely replaced the genus *Agriotes* at high altitudes, especially in Derbyshire (Roebuck & Bray, 1944). It was mainly the species *Corymbites cupreus* F. which the hill farmers identified as damaging their crops, although it was often thought that *Agriotes* was the only genus of economic importance in lowland areas. The genus *Athous* was also fairly common at high altitudes.

Simple controlled experiments were carried out on two aspects of the wireworm problem: (*a*) to compare the effects of the genera *Agriotes*, *Corymbites* and *Athous* in causing damage to oats (the predominant cereal crop in north Derbyshire), and (*b*) to compare the effect of controlled numbers of *Agriotes* spp. on various cereals.

COMPARISON OF THE CHIEF GENERA OCCURRING IN HILL DISTRICTS

Pot experiments were carried out using 10 in. pots, with the surface diameter of the soil 8 in. The wire-5 mm. in length. The soil consisted of medium loams which were well mixed before being placed in the pots. Chemical analysis indicated that no manurial treatment was necessary.

TABLE 2. *Comparison of damage done to oats by* Agriotes *spp.*, Corymbites cupreus *F. and* Athous *spp.*

	No. of larvae per pot	Population per acre	Plants destroyed as fraction of total	Percentage loss of plants
Agriotes spp.	1	125,000	27/150	18
	3	375,000	42/150	28
	6	750,000	72/150	48
	9	1,125,000	99/150	66
Corymbites cupreus F.	1	As above	14/150	9
	3		33/150	22
	6		59/150	39
	9		71/150	47
Athous spp.	3	As above	7/75	9
	6		2/25	8
	9		11/25	44

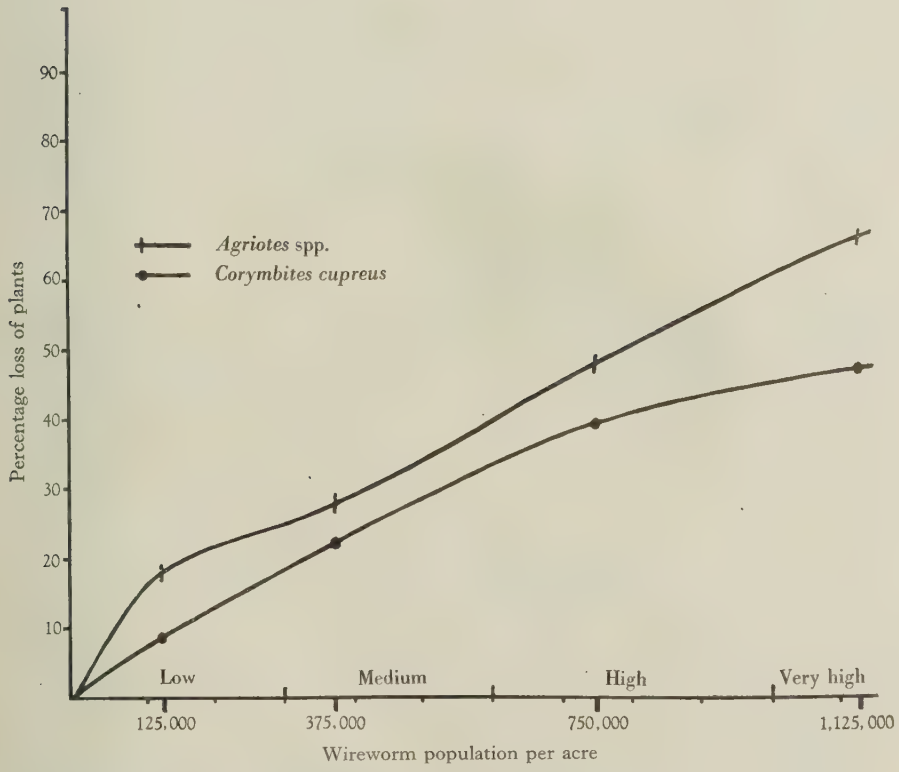

Fig. 1. Percentage loss of oat plants due to larvae of *Agriotes* spp. and *Corymbites cupreus* F.

worm population per acre corresponding to each wireworm per pot was computed to be 125,000. Larvae of different sizes were placed in each pot a week before the seeds were sown; none was under

The seeds were germinated on damp blotting paper and planted as soon as it was seen that they were viable. This obviated the necessity for numerous controls; one control pot, to compare growing

TABLE 3. *Comparison of damage done to cereals by various populations of* Agriotes *spp.*

	No. of larvae per pot	Population per acre	Plants destroyed as fraction of total	Percentage loss of plants	Percentage of unsatisfactory crops (see Table 1)
Winter wheat	3	375,000	30/150	20	24
	6	750,000	42/150	28	32
	9	1,125,000	79/150	53	54
Winter oats	3	As above	40/150	27	—
	6		42/150	28	—
	9		75/150	50	—
Rye	3	As above	27/150	18	17
	6		82/150	55	34
	9		95/150	63	100
Spring wheat	3	As above	48/150	32	—
	6		52/150	35	—
	9		110/175	63	—
Spring oats	3	As above	42/150	28	21
	6		72/150	48	38
	9		99/150	66	59
Spring barley	3	As above	6/125	5	10
	6		28/150	19	41
	9		42/125	34	40

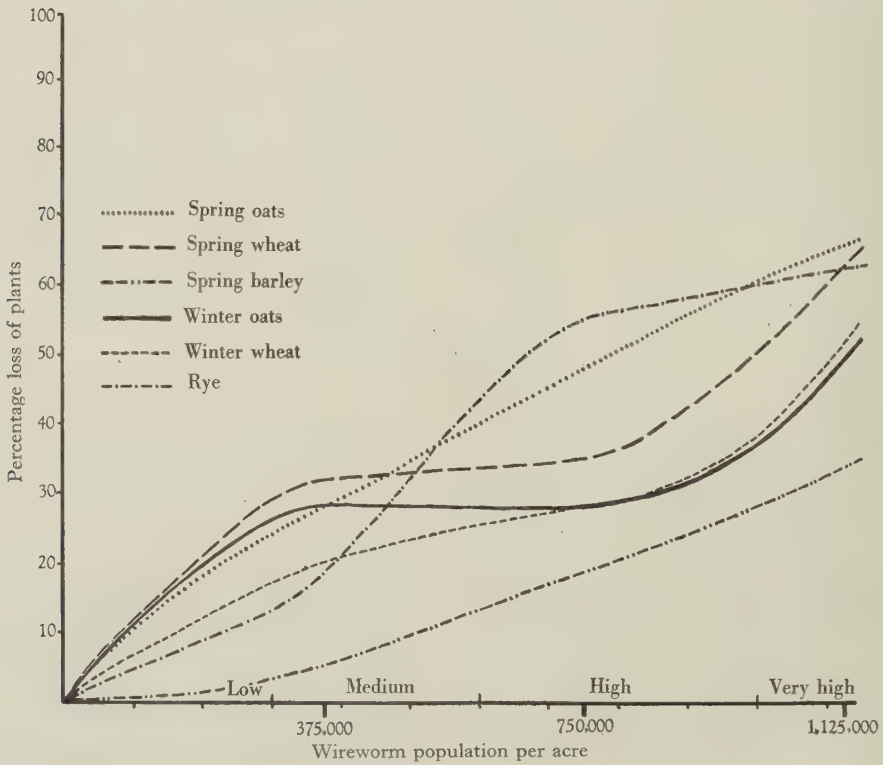

Fig. 2. Percentage loss of plants of cereals due to *Agriotes* spp. larvae.

conditions without wireworms, was included in each experiment. The seeds were sown in a single row of twenty-five across a diameter of each pot. Where possible, six pots (i.e. 150 plants) were used for each crop at each level of wireworm population. They were placed in a heated conservatory and watered fairly frequently. Spring cereals were grown in the spring, winter crops in the late autumn.

Table 2 and Fig. 1 give the number of available oat plants, the number of plants destroyed after 5 weeks' growth and the percentage loss of plants with varying populations of *Agriotes*, *Corymbites* and *Athous* larvae. It is shown that *Corymbites cupreus* F. did damage equivalent to that done by *Agriotes* spp. to oats. Though the data are inadequate it would seem that the genus *Athous* might not be so destructive as the other genera. At the time there were few *Athous* larvae available and a total of only five pots could be set up.

As a subsidiary experiment nine larvae of each genus were added to pots of oats of 5 weeks' growth (controls from the first experiment). At the end of one week 48% (12 of 25) of the plants in the pot containing *Agriotes* spp. had been attacked, 36% (9) being already dead. In the case of *Corymbites cupreus* F. 68% (17 of 25) were attacked and 36% (9) dead. *Athous* spp. had attacked only 8% (2 of 25) of the plants and none was dead. This shows that both *Agriotes* and *Corymbites* will attack fairly large plants and inflict similar damage.

Table 3 and Fig. 2 summarize the results of several pot experiments of 5 weeks' duration using similar populations of *Agriotes* spp. against various cereals. The last column of Table 3 gives the percentage of fields recorded as poor or failure in the survey mentioned above. With winter wheat and spring oats these figures are comparable with the percentage loss of plants in the experiments. The figures for rye are based on an insufficient number of fields. The poor barley crops, due to causes other than wireworm damage, are reflected in the figures given.

It is shown that spring oats and spring wheat are liable to greater damage by species of *Agriotes* larvae than are winter oats and winter wheat. Rye is also liable to severe damage, whereas spring barley is able to tolerate relatively high populations. The latter fact is obviously not due to the later sowing which is the usual practice of farmers, since all the spring cereals in the pots were sown at the same time.

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Simple laboratory and field apparatus for the production of accurate line drawings to scale

By L. N. Staniland, A.R.C.S., D.I.C.,
Seale-Hayne Agricultural College, Newton Abbot, Devon

(With 10 Text-figures)

Apparatus is described by the use of which accurate scale drawings may be made of plants or other biological material for the study of growth changes or the development of disease symptoms. The apparatus has other uses, e.g. enlargement of drawings, etc., and these are also described. Examples of drawings made with the apparatus are included.

Introduction

It is known that the artist Holbein used a tracing method, employing a sheet of glass, in making the portrait drawings in the Windsor Castle collection. Since that time the method has frequently been employed by artists, particularly as a means for the study of perspective. In the experience of the writer this information is not at all widely known amongst biological workers, and there appears to be no record of the use of such apparatus in the course of biological research. Whilst there is little that is new in the principles involved, the writer is confident that the use of such apparatus opens up fresh fields for biological workers; modifications of the apparatus to enable plants to be drawn *in situ* in the field and the incorporation of a lens so that small plants, etc., can be drawn widens the scope considerably.

The apparatus will primarily be considered as a means of making accurate scale drawings of plants at suitable intervals of time, thus securing an accurate and permanent record of the development of symptoms of plant diseases and insect-pest attack; other uses will be mentioned later.

The camera would appear to be the obvious instrument for such purposes, but the drawing method has definite advantages which are listed below:

(*a*) The drawing method is much quicker, when the time taken for development and printing of photographs is taken into consideration.

(*b*) The worker carrying out the drawing exercises his professional powers of selection according to the information he wishes to record and draws only what he requires.

(*c*) There is no difficulty as regards special lighting and it is, in practice, far easier to record the relevant details in shadow than in photography.

(*d*) Difficulties in obtaining depth of focus with plants near to the camera are familiar; this does not arise with the drawing method.

(*e*) The drawing method does not involve skilled draughtsmanship and the method can be used by those who cannot draw freehand. First-class photography requires expensive apparatus and much skill.

(*f*) The apparatus may be kept in the laboratory ready for use at a moment's notice, whenever required. It is cheap and easy to make and inexpensive to use.

(*g*) In common with photographs, it is possible to make measurements on the drawings of lengths in a plane parallel to the glass.

The principles of the apparatus

The simple principles involved are shown in Fig. 1. A sheet of clear glass, *G*, is set up vertically. A small

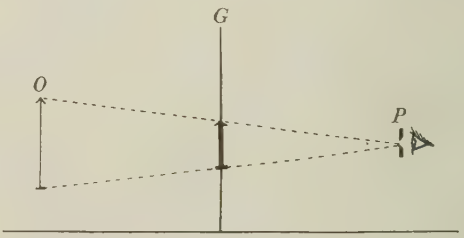

Fig. 1. The principle of the drawing apparatus. *O*, object; *G*, glass; *P*, peep-sight.

'peep-sight', *P*, is placed on one side of the glass and the object, *O*, is placed on the other side. On looking through the sight the object is seen and its outline can be accurately traced by means of a pen and Indian ink on the surface of the glass. The object of the sight is to avoid movements of the eye resulting in effects of parallax.

The extent to which the drawing is reduced from the natural size of the object depends on the distance *PG* in relation to the distance *GO*. Whenever *PG* = *OG* the reduction will be half size. An increase of *PG* gives a larger drawing; a reduction of *OG* also gives a larger drawing. It is clearly not possible to obtain a drawing natural size except in the case of objects in one plane placed next to the glass, when the result would clearly be the same as that obtained by the use of ordinary tracing-paper methods. In practice, drawings five-sixths normal size are about the largest that can usually be made.

Fig. 2 sets out in graphical form the distances of the object and the eye from the glass in order to obtain a number of useful reductions.

The principle involved when a lens is incorporated is shown in Fig. 3. The convex lens *L* is placed close to the glass on the same side as the object *O*. The object is placed at a point just inside the focal length of the lens. On looking through the sight *P* the erect virtual image *V* is seen, and it is this virtual image which is then traced on the glass. Successful drawings up to a magnification of × 2 can easily be made. Different magnifications are obtained mainly by altering the distance *PG*.

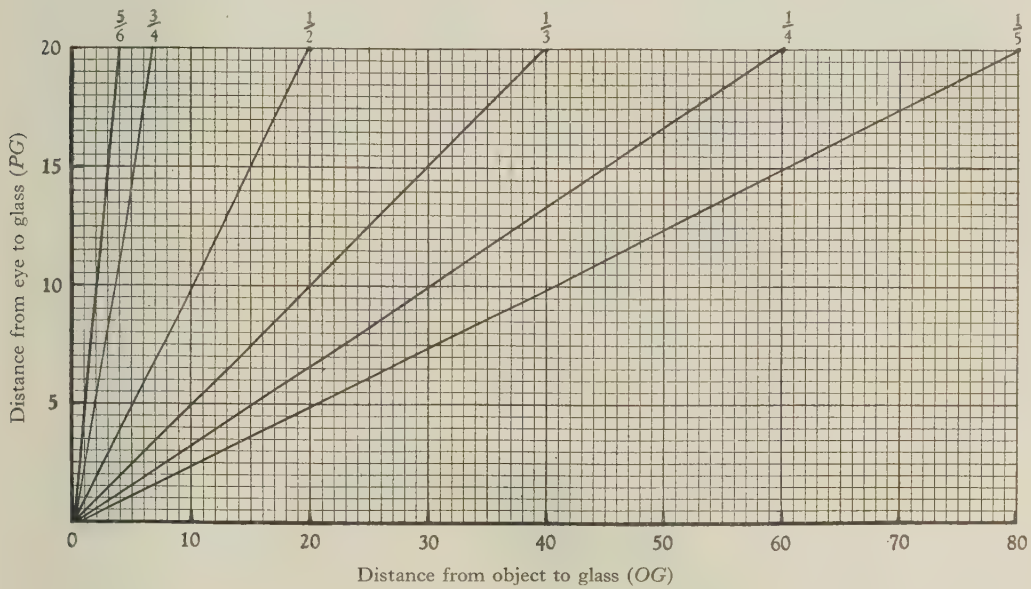

Fig. 2. Diagram setting out the relation between the size of drawing and the distances of the object and the eye from the glass.

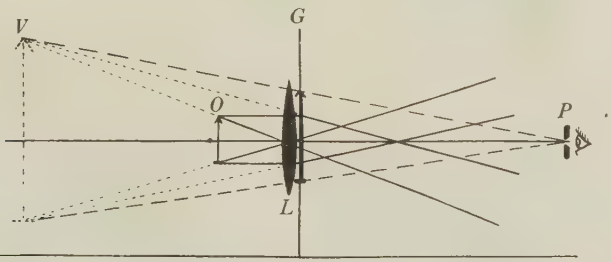

Fig. 3. The principle of the drawing apparatus when a lens is employed.
O, object; *L*, lens; *G*, glass; *P*, peep-sight; *V*, virtual image which is actually drawn.

Construction of apparatus

(1) *For use in the laboratory* (Fig. 4A)

A firm wooden frame is constructed with grooved sides so that a sheet of clear glass about 2 ft. square

can slide in the grooves. Two small wedges can be used to keep the glass quite steady. This frame is set up firmly in a vertical position with a board at right angles on which the object *O* to be drawn can stand. A removable board *t* is provided on which the lens, held by a retort stand or other suitable means, can be placed and easily moved in various directions. The peep-sight *p* is also held in a retort stand for similar reasons. The position of the lens close to the glass when in use is indicated at *l*.

A convenient form of sight is shown in Fig. 4B. The sight is a strip of zinc or tin plate bent as shown. The sight is mounted on a small block of wood of a shape to be gripped firmly in the retort stand. The sight is shown as seen from the side nearest the glass. The bending of the strip brings the actual peep-sight well away from the arm of the retort stand.

The sight is shown in section in Fig. 4C. The hole is $\frac{1}{10}$ in. in diameter at its smallest dimension and is countersunk on the side farthest from the eye, so that any reflexions are directed away from the eye. The whole is painted on all surfaces with matt black paint. In this way disturbing reflexions are finally removed.

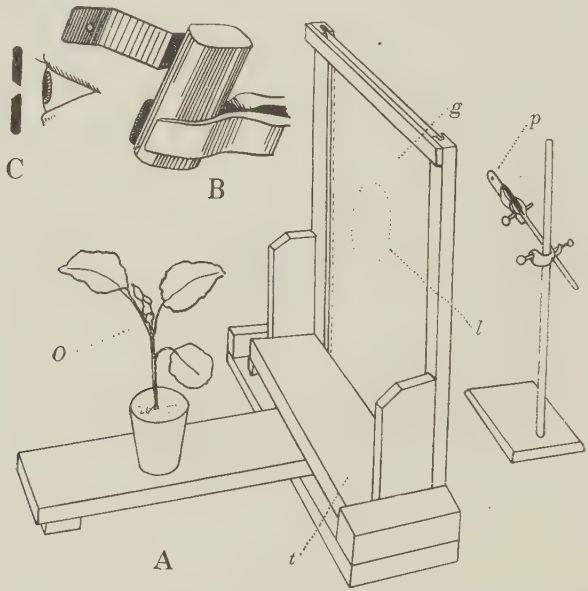

Fig. 4. Construction of drawing apparatus for use in the laboratory. Explanation in text.

(2) *For use in the field*

It is not always possible, or desirable, to remove plants under study into pots with their resulting artificial conditions. The apparatus depicted in Fig. 5 has been found to be very satisfactory for field use.

The glass *g* is supported in a grooved frame as before. Attached by wing-nuts to the base of this frame is a pair of wooden distance-legs *d*, provided with a number of holes of known distance apart, so that the legs can be adjusted and hold the glass at the required distance from the object *O*. A board is also attached to the base of the framework and this bears the sight *p* mounted on a wooden standard. The strip of metal through which the sight is drilled can be adjusted for height by wing-nuts and the position of the sight along the board can also, if desired, be adjustable. In practice it has been found convenient to keep the distance *pg* constant in this field apparatus, the distance from the glass being that found most suited to the sight of the worker. The size of the drawing is then adjusted entirely by altering the distance of the glass from the object.

Underneath the board bearing the sight, and near the end, is constructed an open-sided box *b* into which fit a series of shouldered inclination boards *i* of different lengths. These inclination boards regulate the degree of inclination of the apparatus. If it is desired to make drawings of objects from immediately above, a stake can be driven into the ground and the apparatus tied temporarily to the stake in a vertical position; or a frame specially constructed for such a position can be easily devised.

Attached to the upper surface of the board bearing the sight is a piece of wood *a* to act as an arm rest

whilst drawing. This projects to the right or left according to the hand normally used for drawing.

MATERIALS FOR THE MAKING OF DRAWINGS

The materials are few. Indian ink is used to draw on the glass and the same ink is used for tracing the drawing from the glass to tracing paper. Coloured fixed inks can also be used to draw certain features of an object for purposes of emphasis.

The pen used for drawing on the glass should be fine with a turned up point. In addition to pen and inks, the only requirement is a supply of ordinary tracing paper.

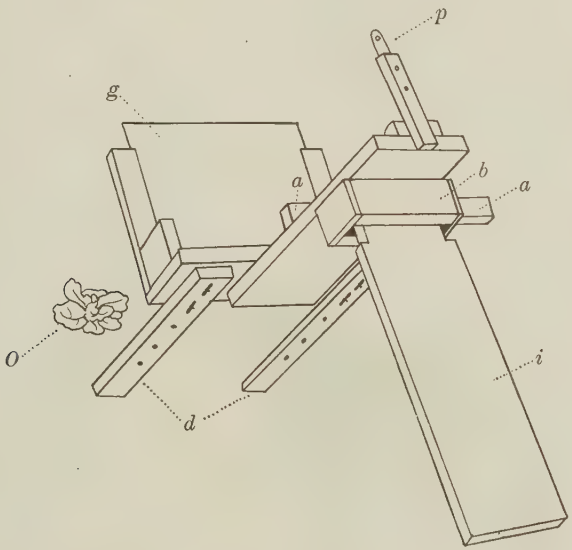

Fig. 5. Construction of drawing apparatus for use in the field. Explanation in text.

To clean the glass when a drawing is finished a cloth moistened in methylated spirit is satisfactory and this leaves the glass in good condition for the next drawing.

THE METHOD OF USING THE APPARATUS

(*a*) *Laboratory apparatus*

The sight and the object are so arranged as to give the reduction required. A millimetre scale may be placed next to the object; the scale is drawn with the object and affords a simple means of calculating the exact reduction. The best viewpoint of the object for the purpose is selected, and the distances of the eye and the object are recorded, also the height of the sight. A mark is made on the object so as to be able to reproduce the viewpoint. Any future changes can be recorded as so many degrees rotation right or left of the previous position, the object being rotated accordingly. Extra lighting may be used if desired to illuminate certain portions if a suitable background is placed behind the object. In tracing round the outline of the object the pen is held where possible in such a position that the nib is pointing slightly downwards and support for the hand is obtained by resting the little finger on the glass. For the upper parts of the drawing the arm may be rested on several books and the pile reduced as the drawing proceeds downwards. For most purposes an outline drawing gives all that is required, but simple shading may readily be introduced where required to an extent commensurate with the powers of draughtsmanship of the worker. When the drawing is dry the sheet of glass is removed from the apparatus and supported in a slightly inclined position over a sheet of white paper on the table. A piece of tracing paper is then laid on the glass and the drawing retraced. The drawing is then in a form where it can be transferred to ordinary paper if a drawing is required for reproduction. Full information, including the recorded distances already referred to, are written on the tracing. Whilst this tracing is being made the object is still in view, undisturbed, for reference. Certain portions of the drawing may be made in coloured inks or certain parts may be coloured in with washes. Shading can also be introduced at this stage.

Where a lens is employed the procedure is similar to that described above. A suitable lens for the purpose is a reading glass of $4\frac{1}{2}$ in. diameter with a focal length of about 9 in. The centre of the lens and the sight should be so adjusted that they are in line with the centre of the object to be drawn. With small objects a $4\frac{1}{2}$ in. lens will enable the whole of the object to be covered at once, though as far as possible use should not be made of the edge of the lens owing to distortion.

Where it is not possible to cover all of the object with the lens it is still possible to make an enlarged drawing of the whole of that object. As much as possible of the object is first drawn and the lens is then raised, lowered or removed to one or other side, keeping in the same plane, so as to cover the next portion. The sight is moved in the same direction until the edge of the drawing first made is seen to register correctly with the image of the object. This is repeated until the complete drawing has been made. Successful drawings of objects twice the diameter of the lens have been made in this way.

(*b*) *Field apparatus*

This apparatus is used in a similar manner to that in the laboratory. Absence of rain is obviously essential and much wind is prejudicial to good results. A screen round the object can be used to prevent movement in a slight breeze. A lens can be held against the glass in a simple holder and be used as already described. The viewpoint having been decided upon, two flat pegs are pushed into the ground so that the distance arms can rest against them. The distance of the glass from the object is decided, also the inclination board to be used, and both recorded. On subsequent occasions it is then possible immediately to place the apparatus in the same position, the registering pegs being left *in situ*. Several sheets of glass may be taken into the field and the tracings on paper made in the laboratory on return.

Whilst the drawing is made the worker either kneels or sits on the ground according to the inclination of the apparatus.

Note. In using either apparatus the best distance of the sight from the glass depends to some extent on the vision of the worker, in particular his least distance of distinct vision. Some workers may prefer about 9 in., whilst those who are long sighted may find a working distance of 18 in. more convenient. Where spectacles have to be worn the sight hole should be made a little larger.

Uses to which the apparatus may be put

As already stated, the primary use of the apparatus is to record at intervals the development of disease symptoms or the effects of insect attack. It may be mentioned here that one tracing may be superimposed on another and when held up to light enable small changes to be more readily appreciated; or composite drawings may be made as shown in Fig. 6. The writer feels that there is a need for more work of this character. Disease symptoms are frequently described in great detail, but not always is their detailed development dealt with. It is far from easy to memorize the sequence of changes, sometimes slight, and such changes can only properly be studied

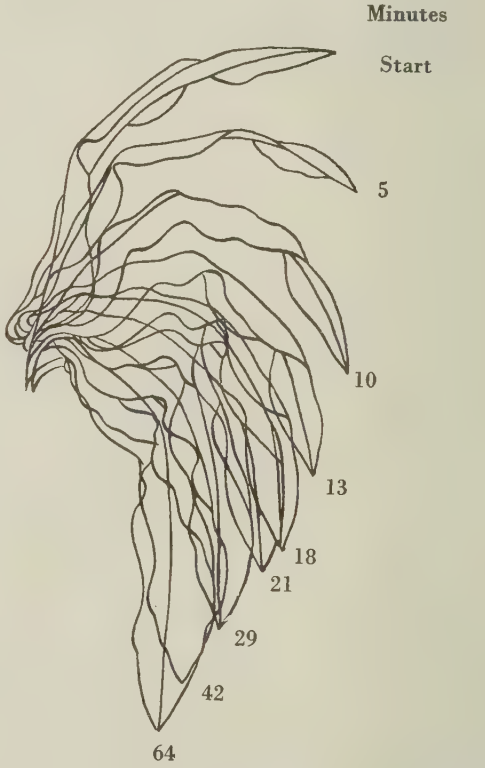

Fig. 6. Outlines of a wilting leaf during 64 minutes superimposed to form one diagram.

by frequent records in concrete form. From a large series a final selection can then be made and put on permanent record.

Natural growth changes or natural normal life movements may also be recorded, and the method also lends itself to portraying the effects of fertilizers and cultural operations. More rapid changes in plants such as take place during wilting have been satisfactorily drawn, so long as movement is sufficiently slow to enable one outline to be drawn before any great change takes place. Such outlines may be superimposed at intervals on the glass and the whole

traced on to paper together (Fig. 6); or separate outlines may be made (Fig. 7). In Fig. 8 the successive positions of the mid-rib only, traced from a series of drawings of another wilting leaf, have been transferred to squared paper. Dotted curves and broken lines have been added connecting the positions of the point of the leaf and the highest points of the mid-rib at the various times. This suggests the possibility of using such drawings for the mathematical study of plant movements.

It is also clearly a simple and accurate means for all workers for the making of simple single drawings of subjects; there is the added advantage that on one sheet of paper can rapidly be recorded a number of viewpoints, e.g. from four points of the compass and from above, thus giving a clear and accurate picture at one glance from many points of view (Fig. 9). It is hoped that this paper may lead many more workers to make an increasing use of simple line drawings with a consequent increase in the value of their papers. Many other uses will suggest themselves but it may be mentioned that geologists would find the method useful, for the recording of such things as geological stratification, faults, etc., and scenic contours are readily made. By tilting the glass at a pronounced angle to the line of vision through the sight (away from the eye) distances in the vertical plane are exaggerated, those in the horizontal plane remaining unchanged.

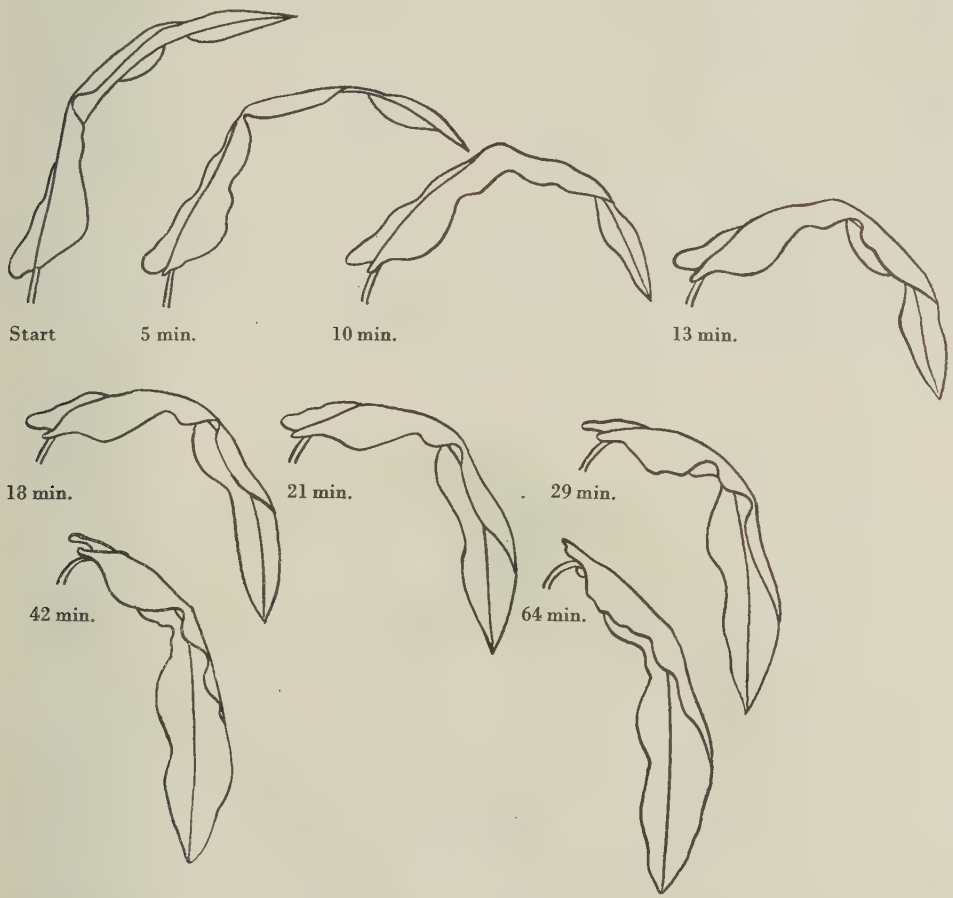

Fig. 7. The same outlines of the wilting leaf as shown in Fig. 6, but set out as separate drawings for purposes of comparison.

The apparatus has a definite place in the laboratory for the reduction of maps and diagrams, and for drawings of apparatus*. It will be appreciated that

* It may be noted that Fig. 4 was drawn with the aid of the apparatus shown in Fig. 5 and vice versa.

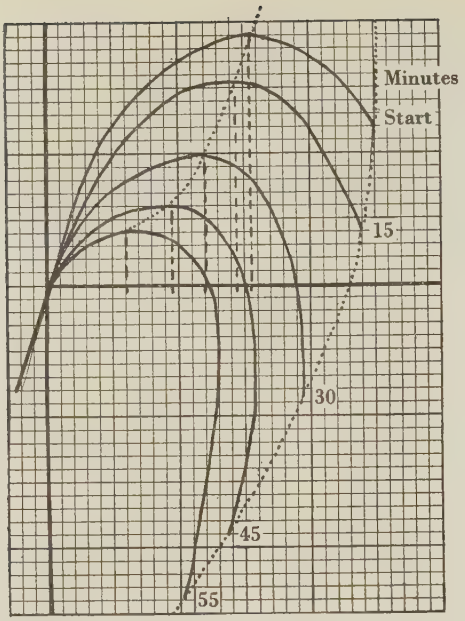

Fig. 8. The lines of the mid-rib of a second wilting leaf, during 55 minutes transferred to squared paper.

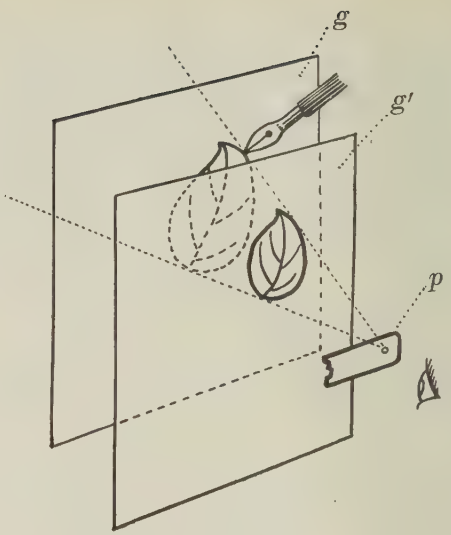

Fig. 10. Diagram showing the use of the apparatus for the enlargement of drawings.

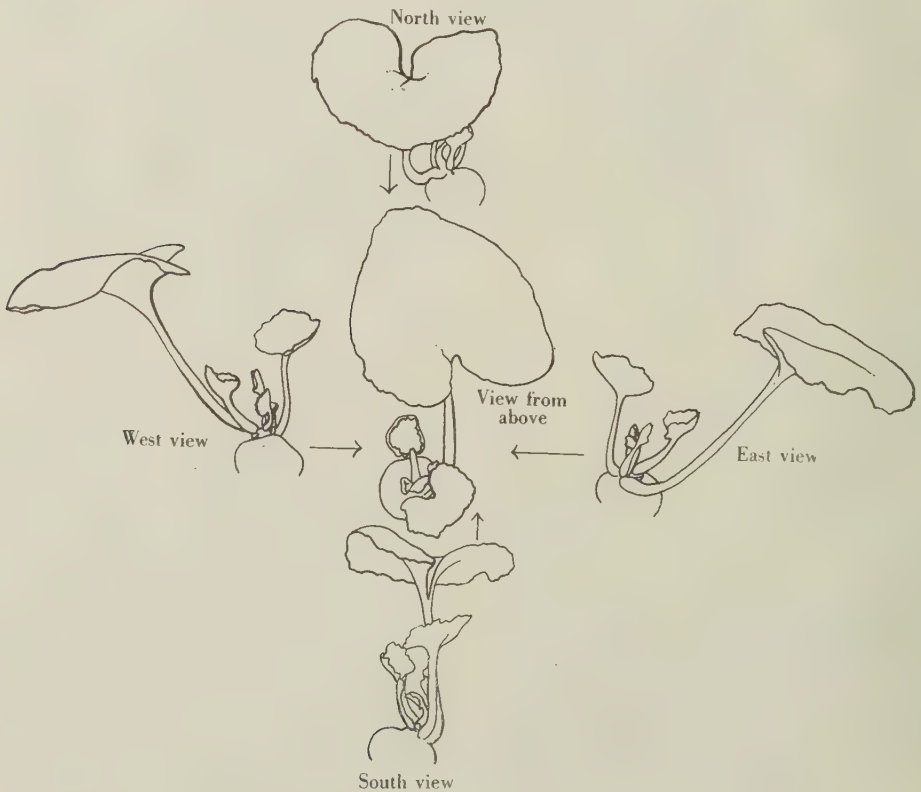

Fig. 9. Five views of a cyclamen plant suffering from a dwarfing disease.

reductions of relief map models can also be carried out; here again a selection of just those features required can be made and features from one map or diagram can easily be transferred to another map or plan, even if the two original maps are on different scales.

Enlargement of maps, drawings or diagrams is also simple. The method is shown in Fig. 10. A second grooved frame with sufficient base to stand firmly is made, the size of the glass being the same as that in the laboratory apparatus. By using the glass as tracing paper the drawing to be enlarged is traced in ink on to this second sheet of glass g'. The glass g' is placed between the peep sight p and the glass of the original apparatus g and parallel to it. A sheet of white card is placed behind the glass g to reflect light.

It will be found easy, with a little practice, to draw on glass g behind the lines on glass g', keeping the point of the pen continuously and accurately hidden behind the lines on g'. The drawing on glass g' should be as near to the edge as possible to facilitate the hand reaching the glass g. The degree of enlargement required can be arrived at by drawing two parallel lines on glass g to equal the height of the drawing required. By moving glass g' and the sight p backwards and forwards the drawing on glass g' (to be enlarged) can be registered against these lines and the enlargement then drawn as described.

The various distances can easily be worked out on squared paper. Taking the position of g' as constant, as g moves away from g' the enlargement becomes greater; as p moves nearer to g' the enlargement also becomes greater.

Examples. When $gg' = 12$ in. and $g'p = 12$ in., the enlargement obtained is $\times 2$.

When $gg' = 12$ in. and $g'p = 6$ in. the enlargement is $\times 3$; but a distance of 6 in. from the eye may be too close to see clearly, so the alternative is to make $gg' = 18$ in. and $g'p = 9$ in. which gives the same enlargement of $\times 3$. In such cases a long-handled penholder is an added help.

(*Received* 18 *October* 1945)

A note on results from spectrographic analysis of coffee material

By C. A. THOROLD, *Department of Agriculture, Trinidad; formerly Plant Pathologist, Department of Agriculture, Kenya Colony*

In the early stages of investigations into the coffee disease known in Kenya Colony as Elgon dieback, it became evident that this disease was not directly associated with fungus attack (Thorold, 1935), and therefore could not profitably be treated by a purely mycological study. Later, it was established that the disease was a physiological one (Thorold, 1945). In the meantime it was presumed that some feature of the environment, either soil or climate, was predisposing the coffee trees to the disorder which culminated in the Elgon dieback symptoms.

It seemed desirable that the possibility of Elgon dieback being a deficiency disease should be investigated. Consequently, with the financial assistance of Major T. H. E. Jackson, now gratefully acknowledged, samples of stems and of beans have been analysed spectrographically.

The samples were sent to Messrs Adam Hilger Ltd., London, who carried out the analyses. The samples submitted consisted of: (1) lateral branches taken at random from two *C. arabica* trees at Kapretwa Estate, then severely affected by Elgon dieback; (2) similar lateral branches taken at random from two *C. arabica* trees, at Kapretwa Estate, then free from dieback, and of the type known to be resistant to this disease (Thorold, 1945). These samples were oven dried before consigning for analysis.

It was possible that comparison of resistant and susceptible material from neighbouring trees might not alone reveal the deficiency sought for, consequently a sample of coffee beans from Kapretwa Estate was sent for analysis, together with a sample of better quality beans having very good appearance and excellent liquoring qualities. It seemed a reasonable supposition that this coffee had come from trees with no nutritional deficiencies, although it was not known for certain that the good quality coffee had been produced in an area where Elgon dieback did not occur.

The two samples of coffee wood were examined qualitatively by means of a medium Hilger quartz spectrograph, exploring both the visible and the ultra-violet regions of the spectrum. There were

certain quantitative indications obtained by inspection and having no actual quantitative value:

Elements present in equivalent amounts in resistant and in susceptible wood: aluminium, barium, calcium, copper, potassium, lithium, manganese, sodium, strontium.

Elements present in greater amounts in resistant as compared with susceptible wood: boron, iron, phosphorus, lead, rubidium, silicon; zinc.

Elements present in resistant wood, but absent from susceptible wood: silver, chromium, nickel, tin.

The coffee bean samples were examined qualitatively and quantitatively, using a medium Hilger quartz spectrograph. The quantitative determinations were made by the arc method of the ratio quantitative system, as described by Judd Lewis (1932). There were no large differences between the two bean samples as regards the major components, of which the amounts present were as follows, expressed as percentages of the air-dried coffee beans: potassium 1·1 %; calcium 0·2 %; magnesium 0·2 %; sodium 0·02 %; rubidium 0·02 %; silica 0·1 %; phosphate 0·4 % to 0·5 %.

In the cases of several of the minor components, the amounts differed quite considerably in the two samples, as shown below.

Minor components expressed as parts per million in air-dried coffee beans

	Good-quality beans	Poor-quality beans
Aluminium	0	30
Barium	5	24
Boron	4	5
Chromium	0·3	0·2
Copper	20	32
Iron	26	60
Lead	0·6	0·2
Manganese	20	27
Strontium	15	20
Silver	0·02	0

The quantitative analyses of stem material show in general a larger amount of the majority of the elements in the more robust, resistant type of material, but no very large differences were revealed except in the case of zinc. This element was not found in the spectra of the bean samples, but its relative abundance may not be important. Arising from this observation, however, some experiments were made with the application to coffee trees of zinc in solution by the 'Roach injection method' (Roach, 1934). No evidence was obtained in regard to increased vigour or disease resistance as a result of the intake of zinc sulphate by these trees. It was concluded that Elgon dieback cannot be accounted for by a deficiency of any of the major or minor elements as determined spectrographically.

The analyses of coffee beans have indicated only excesses of certain relatively unimportant elements (aluminium, iron, and barium) in the poor quality coffee as opposed to the good sample. As regards the important elements, calcium, potassium, magnesium, and phosphorus, a remarkable similarity is shown between the analyses of different bean samples, suggesting that quality differences are not directly associated with the relative abundance of these elements in the beans. On the basis of these results, there would appear to be no scope for the direct improvement of coffee quality through the addition of artificial manures to the soil, although there are many ways in which such manures may affect quality indirectly. The excesses of certain minor elements in the poorer quality of coffee may be indicative of some adverse soil character which could be corrected.

The coffee disease investigation, of which this study forms a part, was undertaken at Kapretwa Estate through the generous co-operation of the owner Major T. H. E. Jackson. Grateful thanks are due to Prof. F. Hardy, Imperial College of Tropical Agriculture, and to Mr J. C. Muir, Director of Agriculture, Trinidad, for helpful criticism and advice.

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The ecology of the larger fungi

V. An investigation into the influence of rainfall and temperature on the seasonal production of fungi in a beechwood and a pinewood

By W. H. Wilkins and G. C. M. Harris,
Mycology Laboratory, University Department of Botany, Oxford

(With 4 Text-figures)

The present paper deals in a somewhat generalized way with the influence of environmental factors, particularly rainfall and temperature, on the seasonal production of fungus sporophores in a beechwood and pinewood, both situated near Newbury (Berks.), during a selected part of the seasons 1933–6, and is intended to be complementary to the previous papers in the same series.

Introduction

This work extends the investigation into the ecology of the larger fungi which was initiated by the senior author in 1933 and carried on until the outbreak of war, when preoccupation with matters of greater national import interrupted the series. The facts were collected by the junior author under the direction and supervision of the senior author, who alone is responsible for their organization and presentation as a continuation of the previous work (Wilkins *et al.* 1937, 1938, 1939 and 1940).

It is a matter of observation and record that environmental conditions, particularly rainfall and temperature, have a marked effect on the time of appearance and numbers of fungus sporophores. The relation between these conditions and the fungi has already been established for grasslands (Wilkins & Patrick, 1940), and the present work attempts to deal with woodlands in a comparable way. Woods which were suitable for the purpose and near enough to Oxford to allow of continued observation were found at Newbury and, by kind permission of the owner, G. E. H. Palmer, Esq., these were examined during the years 1933–6, of which period only one year is represented here.

Previous literature

The literature on the geographical and ecological distribution of fungi has been summarized briefly by Wilkins *et al.* (1937). The only account which has appeared since then is a very brief paper by Lind (1940). She gives a short list of the fungi found during seven visits to certain woodlands, and relates the species to the type of ground vegetation such as *Deschampsia*, *Holcus* and *Pteridium*. She found that the total number of individual fungi was greatest in *Deschampsia*, which also indicated a preference for woods of the 'mor' type. This paper may be regarded as suggesting a line of action whereby Natural History Societies, etc., could compile data which would contribute to a knowledge of fungus ecology.

Ecological notes on the woods

Though both woods were kept under general observation, detailed records are confined to a permanent quadrat of 100 sq.yd. marked out in a specially chosen and representative part of each wood.

(*a*) *The beechwood.* This was situated on the Upper Cretaceous near Hampstead Norris. It was a Plateau beechwood (Watts B type) with mature *Fagus sylvatica*, d.; younger *Fagus*, *Betula pubescens*, *Quercus sessiflora* and *Ilex*, o. Where the canopy was less dense were found *Corylus*, *Ligustrum* and *Rubus* with a field layer of *Mercurialis*. In the denser areas there was a prevernal flora of *Scilla*. The quadrat contained a pioneer beech in the south-west corner which shaded the whole of it. The soil profile showed about 3 in. leaf litter, 3 in. of raw humus merging into humus stained clay-with-flints for a further 6 in., and below this, heavy soil with chalk fragments which increased in size, down to 2 ft.

(*b*) *The pinewood.* This was situated on a ridge of Bagshot Sand above the village of Hermitage. It consisted of old common-land heath with semi-spontaneous *Pinus sylvestris*, d.; *Quercus* and *Betula*, o.; and *Pyrus malus* and *Ilex*, r. The field layer was dominated by *Pteridium* while *Vaccinium* was locally abundant. The less shaded parts had a field layer of mosses and lichens. The quadrat contained two large and two small pine trees and was rather heavily shaded on the south but less so on the north. The *Pteridium* varied from dense to sparse. The soil

profile showed a typical podsol. There was about 3 in. litter, 3 in. raw humus, about 9 in. leached sand and then a 2 in. hard iron and humus pan. Below this was the sand subsoil.

Experimental method

The method mainly involved estimating and recording (*a*) the number of fungi, (*b*) the rainfall, and (*c*) the temperature at each visit. For the sake of comparison these last two were estimated not only on the quadrat but also on a completely unshaded control plot on the open heath and henceforth referred to as the 'Open'. Except in the depth of winter, these three stations were visited approximately once a week throughout the year.

(*a*) *The fungi.* At each visit the fungus sporophores were collected, identified, recorded and destroyed. The intervals between successive visits were sufficiently short to make it unlikely that crops of fungi might appear and die off without being recorded.

(*b*) *Rainfall.* Rain gauges set up, one on each quadrat and one in the 'Open', were read and samples of leaf litter for the estimation of water content were collected at each visit. Two regions of litter were readily distinguishable and separable: (i) the litter representing the most recent autumn leaf fall and designated the 'upper layer'; (ii) the layer below this, referred to as the 'lower layer', and taken to represent the litter of the leaf fall of the previous year. This was indistinguishable from the litter of any previous years, but for our purpose the lower layer has been collected about 3 in. below the surface. The litter samples were taken to the laboratory and, as it had been proved that desiccation to a constant weight and oven drying at 100° C. to a constant weight both gave identical results, the second method was adopted and the water content expressed as a percentage of the wet weight.

(*c*) *Temperature.* The temperature was taken on the quadrats and in the 'Open', by thermometers placed (i) at ground-level, and (ii) at 3 in. below ground-level. The former represented the 'surface' temperature which would act directly on sporophores that were emerging from the mycelial region, and the latter represented the 'below-ground' temperature of the mycelial region where, presumably, the sporophores were being formed. In addition, the maximum and minimum temperatures which had been reached during the interval between one visit and the next were taken at the surface in each of the three stations.

In connexion with both rainfall and temperature it was thought that the canopy might have a significant influence, so the density of the canopy was estimated by a comparison between the light intensity in the woods with that in the 'Open' by means of a light meter. The reduction of light intensity in the woods was assumed to vary directly with the density of the canopy and is recorded as a percentage of the intensity of the light in the 'Open' at approximately the same time.

Results

(1) *The fungi*

Tables 1 and 2 give alphabetical lists of the fungal species collected in the beechwood and the pinewood respectively throughout the season. The number of individuals is expressed in totals per month. As no fungi were found in January, February, March, April or May, these months are omitted from the table.

In both the above woods the fungi show a definite 'season'. No sporophores were found from January to May, but from the start of the season in June there was a steady increase in numbers which reached its peak in October, and then a rapid decrease until December which was the end of the season. The actual numbers of species and individuals are beechwood 35/288 and pinewood 24/4627. The interesting point is that though the pinewood has only two-thirds of the species it has 20 times as many individuals. This high fungus content of the pinewood confirms a statement previously made (Wilkins & Patrick, 1940) that a peak of fungus population is produced not so much by a general increase in numbers of all species as by a very marked increase in numbers of a relatively few species. These species are usually small and delicate and can be produced rapidly on the advent of favourable conditions. The present high figure for the pinewood, for instance, is produced mainly by the species *Mycena galopus*, *M. sanguinolenta* and *Lactarius theiogalus*. Table 3 illustrates this point; only those months in which fungi were found are included in the table.

From Table 3 it will be seen that of the total of 4627 individuals in the pinewood the three species mentioned are responsible for 4266 of them, i.e. 90% of the total. The 'rest' of the species in the pinewood together show a total of 361 individuals which corresponds closely with the total of 288 individuals in the beechwood which, in this particular instance, contains none of these rapidly developing species. This fact confirms the view that there is no direct correlation between number of species and frequency of individuals but, as has often been stressed in this series, the estimation of a mycological flora should take due account of both.

(2) *Environmental conditions*

(*a*) *Effect of canopy on rainfall and temperature*

Perhaps the most significant difference between the conditions affecting fungi growing in the open,

TABLE 1. *Seasonal distribution of fungi in the beechwood*

	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Amanitopsis vaginata	.	.	1	.	.	.	.
Androsaceus epiphyllus	.	.	2	1	2	.	.
Cantharellus cibarius	.	.	2	.	.	.	.
Clavaria pistillaris	.	.	3	1	.	.	.
Clitocybe nebularis	.	.	.	.	1	.	.
C. odora	.	.	.	.	1	.	.
Collybia butyracea	.	.	.	.	46	6	.
C. platyphylla	.	.	.	1	.	.	.
C. radicata	.	.	2	.	.	.	.
Coprinus picaceus	.	.	.	10	10	5	.
Cortinarius brunneofulvus	.	.	.	1	11	.	.
C. hinnuleus	.	.	.	.	14	.	.
Hygrophorus eburneus	.	.	4	3	7	.	.
Inocybe geophylla	.	.	1	.	.	.	.
I. rimosa	.	.	2	.	.	.	.
Lactarius blennius	.	1	4	4	11	.	.
Lycoperdon perlatum	.	.	.	.	5	.	
Marasmius inodorus	.	.	.	2	.	.	.
M. peronatus	.	.	11	6	2	.	.
M. prasiosmus	.	.	.	.	13	5	1
Mycena ammoniaca	.	.	.	.	1	.	.
M. filopes	.	1	.	10	6	1	.
M. galopus	.	.	.	3	4	2	.
M. polygramma	.	.	.	.	1	1	.
M. pura	.	.	1	1	9	.	.
M. sanguinolenta	.	2	.	2	3	.	.
Psathyrella atomata	.	2	2	.	9	5	.
Russula cyanoxantha	1	.	.	.	.	.	.
R. emetica	.	1	1	.	1	.	.
R. fellea	.	.	.	.	1	.	.
R. foetens	.	.	5	.	.		.
R. lutea	.	.	.	.	1	.	.
Scleroderma aurantium	3	.	.	.	.	.	.
Stropharia aeruginosa	.	.	.	.	2	.	.
Tricholoma terreum	.	.	.	1	1	2	.
	4	7	41	46	162	27	1
				288			

e.g. grasslands, and in woods, is the presence of a leafy canopy in the latter. The contemporary effect of a leaf-litter substrate is taken as incidental. The effect of the presence or absence of a canopy on both the amount of rainfall which reaches the substrate and the temperature at substrate level, has been determined by a comparison of factors operating (1) in the 'Open' with no canopy at any time of the year, (2) in the beechwood with full canopy for approximately half the year and no canopy for the other half, (3) in the pinewood which has a canopy all the year round. The month during which the canopy is developing and the month during which it is disappearing, in the beechwood, are omitted.

(i) *Rainfall.* Figs. 1A and B show the relation between the monthly average rainfall in the 'Open' and in the beechwood and pinewood respectively for the 12 months January to December. Rainfall in the 'Open' is regarded as equivalent to the rain which fell on to the canopy in the wood and is represented by the upper graph. The rain which penetrated the canopy and reached the substrate in the woods is represented by the lower graph. Between the two is indicated the relative density of the canopy throughout the year.

From the figure it will be seen that the canopy does affect the comparative amount of rain reaching the substrate. In both woods the amount of rain penetrating the canopy throughout the year, or during any given period in the year, is less than the rainfall outside. As would be expected the beechwood shows considerable variation in rain penetration according

TABLE 2. *Seasonal distribution of fungi in the pinewood*

	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Amanita rubescens	.	.	.	.	4	.	.
Androsaceus androsaceus	.	.	.	1	.	.	.
Clitocybe aurantiaca	.	.	.	14	1	.	.
C. obsoleta	.	.	1	.	6	23	5
Collybia inolens	.	1	.	.	.	.	.
C. maculata	.	.	.	.	2	3	.
Cortinarius mucosus	.	.	.	.	1	2	.
Flammula sapinea	.	.	.	22	28	47	.
Galera hypnorum	.	.	.	1	5	.	.
Hydnum auriscalpium	.	1	8	1	2	14	2
Hygrophorus hypothejus	.	.	.	.	.	1	.
Laccaria laccata	.	.	.	.	1	1	.
Lactarius serifluus	.	.	26	2	24	3	.
L. theiogalus	.	.	.	8	595	107	.
Marasmius conigenus	2	8	2	.	.	5	1
M. peronatus	.	.	3	.	.	.	.
Mycena epipterygia	.	.	.	.	1	1	.
M. galopus	.	13	60	1333	1075	265	22
M. sanguinolenta	21	276	18	305	160	8	.
M. viscosa	.	.	.	.	4	.	.
Naucoria melinoides	.	10	5	.	9	24	9
Nolanea papillata	.	.	.	.	2	10	1
Russula ochroleuca	.	.	5	.	1	3	.
Tricholoma rutilans	.	.	1	.	.	1	.
	23	309	129	1687	1921	518	40

4627

TABLE 3. *The influence of certain species on the total number of individuals*

	Species	Individuals June	July	Aug.	Sept.	Oct.	Nov.	Dec.		
Pine: *Mycena galopus*	.	.	13	60	1333	1075	265	22	4266	4627
M. sanguinolenta	3	21	276	18	305	160	8	.		
Lactarius theiogalus	.	.	.	.	8	595	107	.		
All the rest	21	2	20	51	41	91	138	18	361	
Beech	35	4	7	41	46	162	27	1		288

to whether the canopy is present or absent, and even the pinewood with its permanent canopy shows some variation, though of course less than the beechwood. Assuming the rainfall in the 'Open' to be 100% the effect of the canopy is well illustrated by the following figures. The percentages are approximate:

Rainfall	Open %	Beech %	Pine %
Throughout the whole 12 months	100	70	75
Nov. to Apr., no canopy in beech	100	85	80
June to Sept., full canopy in beech	100	55	70

It is apparent that with no canopy in the beech, 85% of the rain passes through, but with full canopy only about 55% comes through. The pine canopy is always more easily penetrated by rain, and variation between the amount which penetrates the rather more dense summer canopy and that penetrating the rather less dense winter canopy is only about 10%. The kind of rainfall also makes some difference; heavy rain penetrates both types of woods almost equally and to a very high degree; medium and particularly light rainfall is more effectively intercepted by the canopy especially in beech, where there may be considerable evaporation from the leaves.

(ii) *Temperature.* Figs. 2 A and B show the average maximum and minimum temperatures in the 'Open' (outside graphs) compared with the maximum and minimum in the beechwood and pinewood (inside graphs). The shaded part represents the difference in temperature between the 'Open' and the woods, as produced by the canopy, the density of which is also represented.

It is evident that in both beechwood and pinewood there is considerable modification in the range

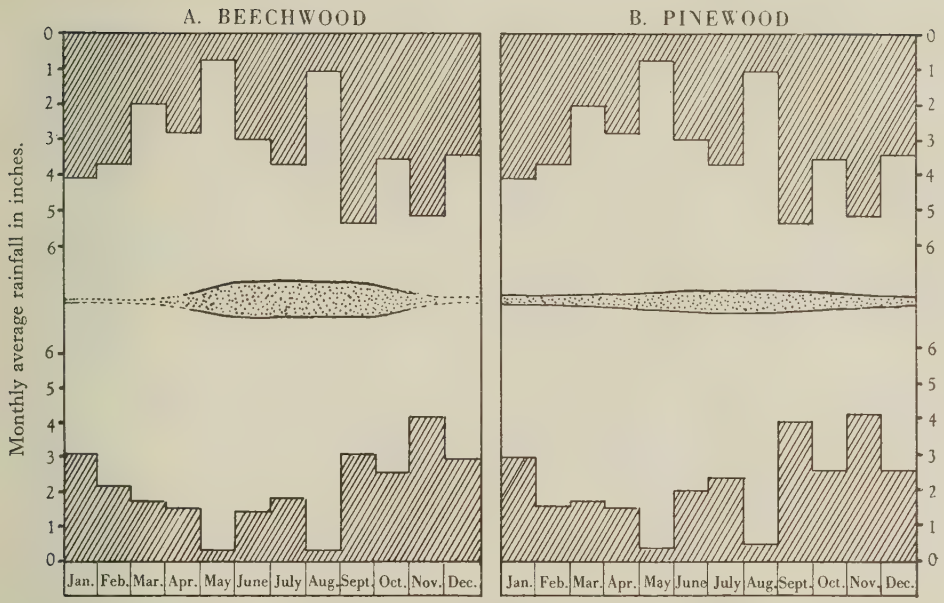

Fig. 1. The upper shaded region shows the average monthly rainfall falling on the canopy (stippled), and the lower shaded region shows the average monthly rainfall reaching the ground in the woods. The stippled strip represents the density of the canopy.

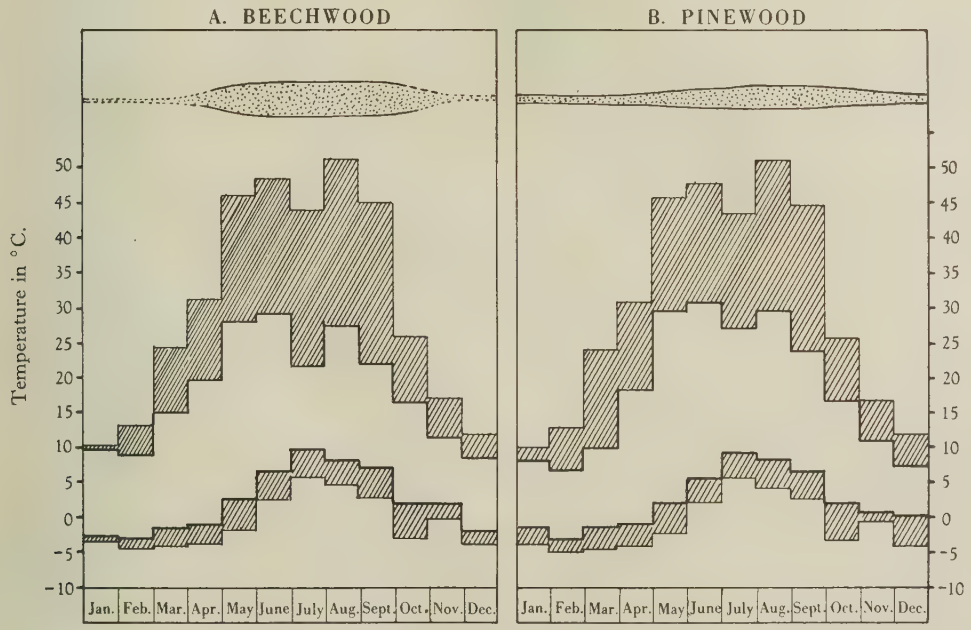

Fig. 2. Showing average monthly temperature range outside the woods (shaded) and inside the woods (unshaded). The stippled strip represents the density of the canopy.

between maximum and minimum in the woods as compared with that in the 'Open'. This modification of the temperature extremes, while affecting both the maximum and minimum, is especially emphasized in the former. The maximum in the 'Open' may go to above 50° C., but in the woods it rarely goes above 30° C. for any length of time. The minimum in the woods is at best about 10° C. less than outside. The higher the maximum temperature outside, the greater is the blanketing effect of the canopy on the maximum temperature inside the wood. That this modification is produced by the canopy is evident, because it is more pronounced in the beechwood when the canopy is dense than when there is no canopy. Similarly, the pinewood whose canopy is permanent but never so dense as the full canopy of beech shows less temperature modification when compared with beech in full canopy but more when compared with beech with no canopy. These points are illustrated by the following figures where the average range between the maximum and minimum temperature in the 'Open' is taken as 100 and the average range in the woods is expressed as a percentage of this:

Average range of temperature	Open %	Beech %	Pine %
Throughout the whole 12 months	100	50	50
Nov. to Apr., no canopy in beechwood	100	65	50
June to Sept., full canopy in beechwood	100	35	45

It seems that the effect of the canopy reduces the temperature range in both the woods to approximately 50% of that in the 'Open'. In the pinewood the temperature is fairly constant all the year round, but in the beechwood the presence of the full canopy reduces the range to 35% of that in the 'Open', whereas in the absence of canopy the reduction is only to about 65%. Even when there are no leaves on the beech there is still some canopy effect, and in the pine there is variation in canopy density throughout the year.

(*b*) *Effect of rainfall and temperature on conditions in the woods*

It is assumed that the water available for the growth of fungus mycelium and the production of sporophores is obtained directly from the substrate. The water content of the substrate, however, varies directly with the amount of rainfall which reaches it. The keeping up of the water content of the substrate depends on its ability to retain moisture, and the retention of moisture depends on many factors of which temperature is the only one dealt with here. Figs. 3A and B show the interrelationship between rainfall, temperature at surface level and at 3 in. below the surface, and the water content of the upper and lower layers of leaf-litter in the beechwood and the pinewood throughout the year. The results are expressed as monthly averages plotted in the middle of each monthly period.

In both beechwood and pinewood there is a close relationship between the rainfall and the water content of the litter, particularly the lower litter. The woods being situated within a mile or two of each other, it is not surprising that the rainfall graphs are very similar, even allowing for the fact that the rainfall in the woods has been affected by the canopy as mentioned above. The graphs of the water content of the lower litter throughout the year are also strikingly similar in both types of wood, but, though plotting monthly averages tends to smooth out the fluctuations, it is obvious that the variation in the water content of the lower litter is a slow and steady process as compared with the relatively violent variation of the rainfall. In both types of wood the water content of the upper litter is much more variable than that of the lower litter, and this (the rainfall being the same) must be attributed to the influence of the surface temperature. The temperature taken 3 in. below the surface, and so corresponding with the position of the lower litter, varies so little that it appears to have no influence on variation of water content. In both the above graphs (Fig. 3) the surface temperature was low in January, but from February there was a steady rise which reached its peak in June. In the beechwood the rise in temperature from February to March was quite significant, and this seems to have had an immediate effect on the upper litter water content which dropped rapidly. From this time until September, with the exception of a slight rise in July due to heavy rain, it remained at this level. After September the rainfall increased while the surface temperature decreased, hence the water content of the upper litter increased till it reached between 70 and 80%, becoming equal to that of the lower litter. In the pinewood the state of affairs is somewhat different, the water content of the upper litter is always relatively low as compared with that of the lower litter. Here also it seems to be influenced by temperature, in that it increases with the fall of temperature and decreases with rise of temperature, but its response to temperature variation is less violent than that of the water content of the upper litter layers of beech.

(3) *Effect of environmental conditions on the production of sporophores*

Apart from composition of substrate, the main environmental factors which affect the production of fungus sporophores in woodlands are water content of the substrate and the temperature. The lower leaf-litter has been chosen as the representative substrate because 3 in. below the surface is a usual place for fungus mycelium and sporophore production. It has been shown that temperature variation is small

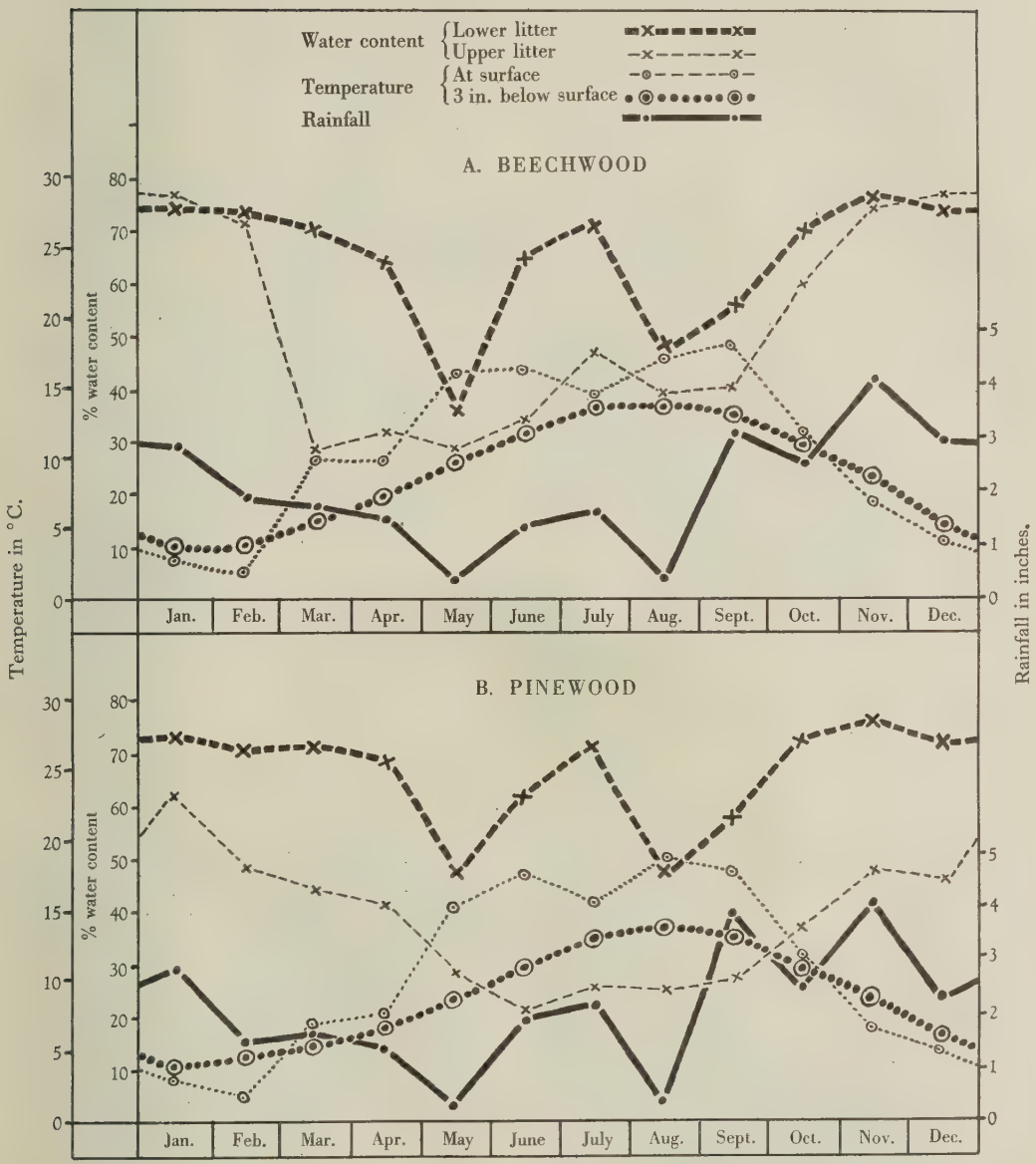

Fig. 3. Relation between rainfall and temperature on the one hand and water content of the substrate on the other.

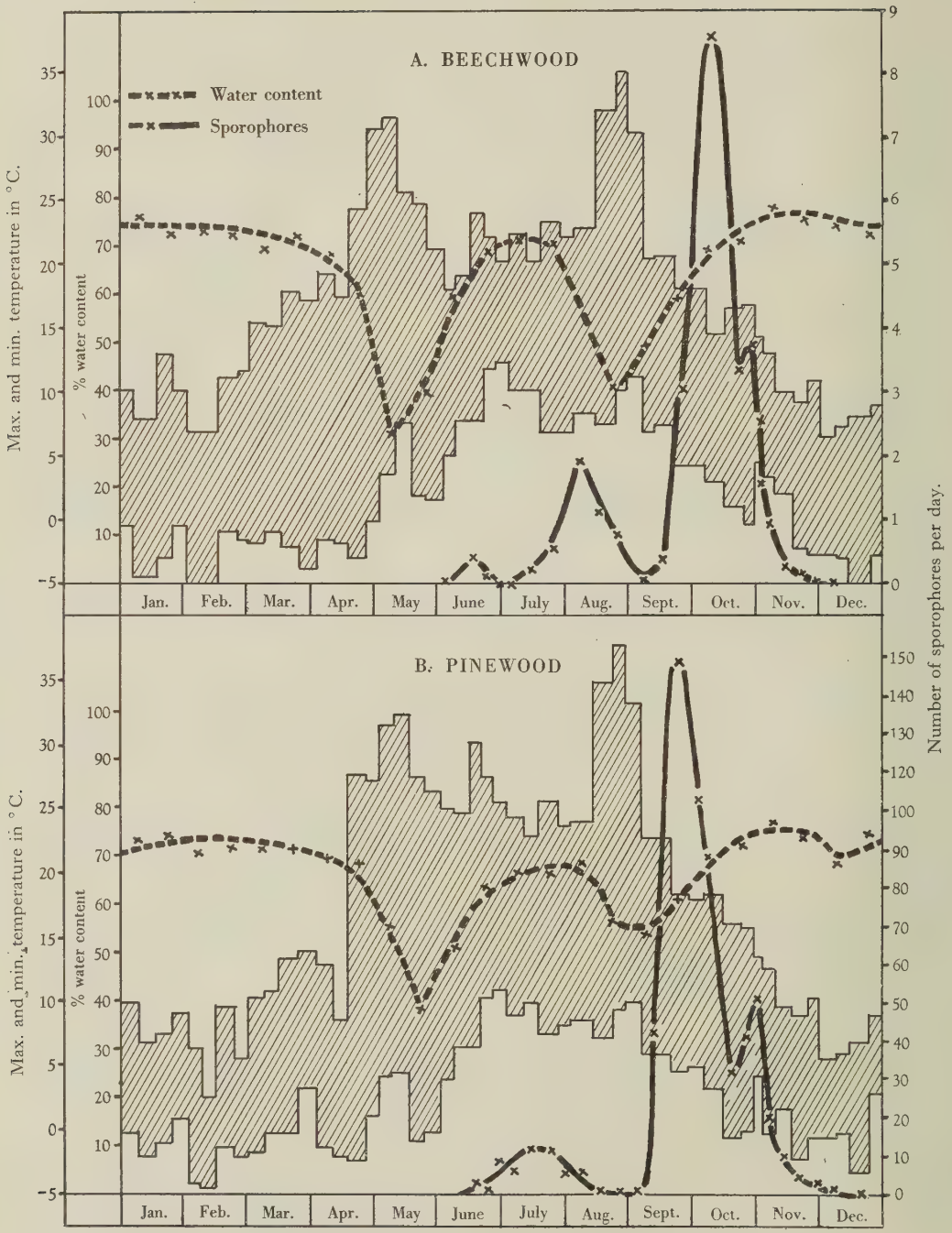

Fig. 4. Numbers of fungi in relation to temperature and water content of the substrate.

at this level and hence has little effect on water loss. This ensures mycelial perennation but does not necessarily enable sporophores to emerge from the substrate. The maximum and minimum temperatures are the most significant because a high maximum and a low minimum are both inhibitory to sporophore emergence, whereas mean temperature, though important, is not in itself sufficiently determinative. The maximum and minimum temperatures were taken at ground-level because the emergence of sporophores takes place there and will not do so if temperature conditions are unfavourable. Moreover, the range of temperature between maximum and minimum is greater at the surface than it is above or below the surface. The relation between the numbers of fungi and the temperature and water content of the substrate throughout the year in each type of wood is shown in Figs. 4A and B.

The graphs for beechwood and pinewood are essentially similar in all respects except for the larger number of fungi in the latter (note different scale). In both cases the highest peak of sporophore production is in the autumn, mainly September–October. There is a similar peak in July–August following, after a lag phase, the increased water content which started in May. The results confirm those previously established, viz. that the production of large numbers of fungi is dependent on favourable conditions of water content and temperature and that these conditions are usually operative to the highest degree only in the autumn.

CONCLUSIONS

It seems to be established for both grasslands and woodlands that the major factors influencing sporophore production are two, viz.: water content of substrate and temperature. The first is sometimes conveniently expressed as rainfall though this is not an exact equivalent. For fungi to be produced in abundance the first must be above a certain level, and the second must be within a certain range, and they must both continue to operate for a given, though variable, period of time. In that both factors must be operative it is hardly reasonable to say that one is more important than the other, but it is nevertheless true that while many species are relatively indifferent to temperature all are dependent on adequate water supply. Similarly, the minimum temperature has more influence than the maximum, for a low minimum will inhibit development directly, whereas a high maximum is more or less indirectly effective as being associated with a low water content in the substrate. It appears probable that, given favourable water and temperature conditions, fungi could be produced at any time of year, and that therefore the rhythmic periodicity of their appearance is not so much a function of time as of conditions. We have estimated that in a beechwood, for instance, sporophores could be produced (i) when the water content is above 50%, and (ii) when the arithmetic mean of minimum temperatures is above 4° C. These two coincide only from the beginning of June to half-way through August, and again from the beginning of September to half-way through October, and these two periods are in fact the only times when sporophores are produced in any quantity. Hence the so-called spring and autumn fungus seasons. The same argument can be applied to a pinewood except that here perhaps the water-content percentage needs to be slightly higher.

The length of time during which the favourable conditions must operate before fungi will appear varies with individual species. There is a lag phase in sporophore production after the onset of what may be regarded as favourable conditions. In general, it can be said that the larger the fructification the longer the lag, hence the beginning of any favourable period is characterized by the appearance of numbers of small species, and only if the period continues for some time will large fructifications be produced. Fungi can be divided into four groups according to their 'lag and duration' reactions. Some, e.g. *Clitocybe aurantiaca*, start early and finish early; some, e.g. *Lactarius theiogalus*, start late but continue to the end; some, e.g. *Hygrophorus eburneus*, have a brief life in the middle of the period; and lastly, some, e.g. *Mycena galopus* and *M. sanguinolenta*, start relatively early and continue for a long time. In all cases there is a peak of sporophore production and obviously the position of the peak in time will vary with individual species. The peak of total production will be a summation of the individual species' contribution and, as we have noted, is usually determined by a few species only. The smaller fungi growing in the upper litter naturally get going more quickly, but rapidly fall off in abundance when the water content of the upper litter diminishes.

Most of the above generalizations apply both to the grassland and to woodland fungi. Woods, however, have certain advantages over grassland because the canopy of the woods may, and often does, exercise a modifying effect on inhibitory influences. For instance, the canopy reduces the amount of rain reaching the substrate, and this is more marked in the beechwood than in the pinewood. This may be a disadvantage, but it is more than compensated by the modification of temperature extremes which is produced by the canopy. In the 'Open' the average maximum temperature during May to September was in the region of 45° C., whereas that in the woods rarely exceeded 30° C. Similarly, the average minimum temperature for the same period was higher in the woods by some 5° C.

On the whole it seems that woods offer a better habitat for fungi than open country in that apart from substrate composition which is not being considered here, the modifying effect of the canopy enables the fungus season to start earlier, continue longer and to be more generally equable all round.

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Investigation into the production of bacteriostatic substances by fungi

Preliminary examination of more of the larger Basidiomycetes and some of the larger Ascomycetes

By W. H. Wilkins, *Mycology Laboratory, University Department of Botany, Oxford*

This paper may be regarded as an addendum to previous work along the same lines, more particularly the work which dealt with some 700 of the larger Basidiomycetes. Both Ascomycetes and Basidiomycetes were tested for their antibiotic properties by means of sporophore extract. Of the ninety-nine fungi tested, twenty-eight showed a positive and seventy-one a negative reaction against the test bacteria, *Bacterium coli* and *Staphylococcus aureus*.

The sixty-five Basidiomycetes and the thirty-four Ascomycetes examined were the larger specimens of the woods and pastures and none of them was grown in culture. The details of technical method are as in the previous paper (Wilkins & Harris, 1944*d*). The sporophore 'juice' was extracted and tested by the method previously described (Wilkins & Harris, 1943*a*). Most of the extracts were sent by my colleagues Messrs E. W. Swanton and A. A. Pearson from Haslemere, Surrey, and to them my best thanks are again due. The extracts were tested for their antibacterial action against the two representative types of bacteria, *Bacterium coli* and *Staphylococcus aureus*, and in the following list a positive reaction is indicated by the symbol '×' and a negative result by the symbol 'o'.

The Ascomycetes with 35 % of the species positive give a relatively higher number of positive reactions than the Basidiomycetes with 25 % of the species positive. As, however, only comparatively few Ascomycetes have been examined these will not be discussed further at present.

Taking this paper in conjunction with the previous one (Wilkins & Harris, 1944*d*), which dealt with Basidiomycetes only, the total number of Basidiomycetes examined to date is 787. These react antibiotically as follows:

Positive against *B. coli* and *S. aureus*	51 = approx.	6 %
Positive against *B. coli* only	14 = approx.	2 %
Positive against *S. aureus* only	98 = approx.	11 %
Negative against both bacteria	624 = approx.	80 %

No indication is given in the list of the *degree* of

List of fungi examined

Ascomycetes

	Bact. coli	*Staph. aureus*		*Bact. coli*	*Staph. aureus*
Acetabula vulgaris Fuck.	o	×	*Helvella lacunosa* Afz.	o	o
Aleuria cevea (Sow.) Gill.	o	o	*Hypoxylon coccineum* Bull.	o	o
A. subrepanda (Cke. & Phill.) Gill.	o	×	*Leotia lubrica* (Scop.) Pers.	o	o
A. vesiculosa (Bull.) Boud.	×	×	*Leptopodia elastica* (Bull.) Boud.	o	o
Bulgaria inquinans (Pers.) Fr.	o	×	*Macropodia macropus* (Pers.) Fuck.	o	o
Calycella citrina (Hedv.) Quél.	o	o	*Microglossum olivaceum* (Pers.) Gill.	o	o
Cordyceps capitata (Holms.) Link.	o	×	*Mitrula phalloides* (Bull.) Chev.	o	o
C. militaris (Linn.) Link.	×	×	*Morchella elata* Fr.	o	o
Coryne sarcoides (Jacq.) Tul.	o	o	*M. esculenta* Pers.	o	×
Daldinia concentrica (Bolton) Ces. & de Not.	o	o	*M. leporina* (Batsch.) Fuck.	o	o
			M. onotica (Pers.) Fuck.	×	×
Dascyscypha luteola (Curt.) Sacc.	×	o	*Peziza aurantia* Pers.	o	o
Elaphomyces variegatus Vitt.	o	o	*P. rutilans* Fr.	o	o
Galactinia badia (Pers.) Boud.	×	×	*Rhizina inflata* (Schaeff.) Karst.	o	o
G. succosa (Pers.) Sacc.	×	×	*Sepultaria arenicola* (Lév.) Mass.	o	o
Geoglossum glutinosum Pers.	o	o	*Spathularia clavata* (Schaeff.) Sacc.	×	×
G. ophioglossoides (Linn.) Sacc.	o	o	*Xylaria hypoxylon* (Linn.) Grev.	o	o
Helvella crispa (Scop.) Fr.	o	o			

Basidiomycetes

Amanita aspera (Fr.) Quél.	o	o	*Hypholoma lachrymabundum* Fr. non Bull.	o	×
Boletus Queletii Schulzer	o	×			
B. sulphureus Fr.	o	o	*Hypochnus echinosporus* (Ellis.) Burt.	o	o
B. tridentinus Bres.	o	o	*H. fumosus* Fr.	o	o
Bourdotia Eyrei Wakef.	o	o	*Lactarius flexuosus* Fr.	o	o
Clavaria umbrinella Sacc.	o	o	*Lentinus lepideus* Fr.	o	o
Clitocybe rivulosa (Pers.) Fr.	×	×	*Lenzites saepiaria* (Wulf.) Fr.	o	o
Collybia inolens Fr.	o	o	*Lepiota constricta* (Fr.) Quél.	o	o
Corticium confine Bourd. & Galz.	o	o	*Leptonia solstitialis* Fr.	o	o
C. (*Gleo.*) *porosum* Berk. & Curt.	o	o	*Merulius rufus* (Pers.) Fr.	o	×
Cortinarius (*Dermo.*) *croceoconus* Fr.	o	o	*Mycena crocata* (Schrad.) Fr.	o	o
C. (*Hydro.*) *balustinus* Fr.	o	o	*Naucoria pediades* Fr.	o	o
C. (*Hydro.*) *detonsus* Fr.	o	×	*N. vervacti* Fr.	o	o
C. (*Hydro.*) *renidens* Fr.	o	o	*Peniophora hydnoides* Cke. & Massee	o	×
C. (*Hydro.*) *subferrugineus* (Batsch.) Fr.	o	o	*P. subalutacea* (Karst.) v. Hoehn. & Litsch.	o	o
C. (*Ino.*) *argentatus* (Pers.) Fr.	o	×			
C. (*Phleg.*) *causticus* Fr.	o	o	*Phallus imperialis* Schulz.	o	o
C. (*Phleg.*) *multiformis* var. *flavescens* Cke.	o	×	*Pholiota erebia* Fr.	o	o
			Pleurotus phlebophorus (Ditm.) Fr.	o	o
C. (*Phleg.*) *porphyropus* (A. & S.) Fr.	o	o	*Polyporus lacteus* Fr.	o	o
C. (*Phleg.*) *turbinatus* (Bull.) Fr.	o	o	*P. rutilans* (Pers.) Fr.	o	×
C. (*Tela.*) *psammocephalus* Fr.	o	o	*P. spumeus* (Sow.) Fr.	o	o
Femsjonia luteoalba Fr.	o	o	*Poria gilvescens* Bres.	o	o
Flammula fusus (Batsch.) Fr.	×	×	*P. xantha* Lind.	o	×
F. fomentarius (Linn.) Fr.	o	o	*P. vaporaria* Vitt.	o	o
Fomes fraxineus (Bull.) Fr.	o	o	*Psalliota straminea* Schaeff.	o	o
Ganoderma resinaceum Bourd.	o	o	*P. xanthoderma* Genev.	×	×
Grandinia Brinkmannii (Bres.) Bourd. & Galz.	o	o	*P. xanthoderma* v. *grisea* Pearson	×	×
			Russula atrorubens Quél.	o	o
Hydnum repandum v. *rufescens* (Pers.) Fr.	o	×	*R. flavo-virens* Bomm. & Rouss.	o	o
			R. gracillima Schaeff.	o	o
Hygrophorus citrinus Rea.	o	o	*R. integra* (Linn.) Bataille	o	o
H. coccineus (Schaeff.) Fr.	×	×	*R. melliolens* Quél.	o	o
H. pratensis v. *pallidus* B. & Br.	×	o	*R. veternosa* Fr.	o	o
H. sciophanus Fr.	o	o	*Stropharia caput-Medusae* (Bull.) Fr.	o	o

positive reaction. This varies from a zone of inhibition 2 mm. wide to one of 13 mm. wide, but it has been found by experience that a fungus extract which gives only slight indication of antibacterial activity is not necessarily less important than one which gives a large zone, as bacteriostatic substances of the former class may be, therapeutically, as significant as those of the latter class.

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The production of viridin by pigment-forming strains of *Trichoderma viride*

By P. W. BRIAN, P. J. CURTIS, H. G. HEMMING AND J. C. McGOWAN,
Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks

(With 3 Text-figures)

Viridin is a highly fungistatic metabolic product of certain strains of *Trichoderma viride*. All viridin-producing strains of this mould so far examined produce a yellow pigment abundantly in culture (Weindling's *P series*), which distinguishes them from the gliotoxin-producing strains which in all recorded cases have been of the non-pigment-forming type (Weindling's *O* and *Q series*).

Cultural conditions affecting production of viridin in liquid media have been studied. A range of sugars and polysaccharides, and glycerol, can be utilized for the production of fungistatically active culture filtrates. Not all nitrogen sources which will support active growth are suitable for viridin production. Ammonium salts or peptone are quite suitable but nitrates are not. It is shown that media containing nitrates show a consistent tendency to develop a high *p*H during the growth of the mould, even where the medium is adjusted to *p*H 3·5 initially. Viridin is known to decompose in aqueous solution at or near neutrality, and it is the characteristic *p*H drift of nitrate-containing media which is probably responsible for their unsuitability for viridin production. There are minor differences in the nutrient requirements of the three strains of *T. viride* studied.

The depth of the medium is of importance in determining the fungistatic activity of culture filtrates. On shallow layers (0·5 cm.) a peak of activity is usually reached about the sixth day of incubation at 25° C., after which activity falls off rapidly. There are marked differences in the drift of activity of culture filtrates of the three strains of *T. viride* studied; strain 213, which produces the most active culture filtrates, shows the most acute peak of activity. Deepening the medium has the effect not only of

increasing the time taken to reach maximum fungistatic activity, and the degree of activity, but also leads to an absolute reduction in the amount of fungistatic material produced.

Viridin may be isolated from active culture filtrates by extraction with chloroform, evaporation to dryness and recrystallization from ethyl alcohol, methyl alcohol or benzene. The crystals produced from each of these three solvents differ in form, but analyses of all agree with the formula $C_{20}H_{16}O_6$. Viridin is soluble in chloroform, hot ethyl alcohol and hot methyl alcohol; it is sparingly soluble in carbon disulphide and carbon tetrachloride, and almost insoluble in ether. It is optically active; for a 1% solution in chloroform $[\alpha]_D^{19}$ is $-222°$. An account is given of some of the chemical reactions of viridin.

Viridin shows a marked degree of specificity in its fungistatic effects. Germination of conidia of *Botrytis allii* is prevented at 0·006 μg./ml., whereas 6·25 μg./ml. is needed to prevent germination of conidia of *Penicillium expansum*. *Trichoderma viride* conidia are insensitive to as much as 50 μg./ml. Higher concentrations are needed to be fungicidal; for *Botrytis allii* a concentration 1000 times greater than the minimum needed to prevent germination is needed to give a complete kill by 2 hr. exposure. Viridin has little if any antibacterial action, and is therefore unique among all antibiotic mould products so far described, as all those previously described showing antifungal activity are also antibacterial.

Aqueous solutions of viridin are not stable unless acid. At *p*H 2·9 half the activity of a solution is retained after 14 days at 25° C., at *p*H 5·8 all activity is lost in 1 day, and loss of activity is immediate at *p*H 8·4. Aqueous solutions of viridin are inactivated by peptone and yeast extract, and by a number of pure chemical compounds likely to be present in such materials.

Introduction

Many examples of antagonism between fungi are now known, but by far the most striking is that exhibited by *Trichoderma viride* to a considerable range of saprophytic and plant-pathogenic fungi. Our knowledge of the antagonistic behaviour of *Trichoderma* is largely due to the pioneer work of Weindling (1932, 1934, 1937, 1941), who first demonstrated the production of a fungicidal substance by this mould. He succeeded in isolating (Weindling & Emerson, 1936) an organic substance possessing fungicidal properties from culture media upon which had been growing a fungus at first identified as *T. lignorum* (= *T. viride*), but subsequently stated to be *Gliocladium fimbriatum*. This substance, gliotoxin, has since been shown to be produced by a number of fungi, including *Trichoderma viride* (Brian, 1944; Brian & Hemming, 1945). More recently it has been shown (Brian & McGowan, 1945) that certain strains of *T. viride* produce another antibiotic substance known as viridin, characterized by very marked fungistatic properties. In this paper results are presented dealing with the production of viridin by *T. viride*, its biological activity, and with some of its chemical and physical properties.

Viridin-producing strains of *T. viride*

The results reported here have been obtained with three strains of *T. viride*, differing in cultural characteristics.

Strain 10: Originally received from Prof. H. Raistrick in October 1942, as *Gliocladium* sp. (culture from C. Thom, 1930).

Strain 213: Isolated in March 1944, from a greenhouse soil at Jealott's Hill.

Strain 214: Isolated in April 1944, from a greenhouse soil at Jealott's Hill.

These strains, like the strain 12 described elsewhere (Brian, 1944), though they have a rather *Gliocladium*-like conidiophore, are typical of *Trichoderma viride* in size of conidia, presence of chlamydospores and rapid growth. Strains 10 and 214 have a diffuse habit of growth on Czapek-Dox agar, and there is little, if any, indication of formation of tufts of conidiophores. Strain 214 produces conidia very sparingly, and cultures appear to be a much paler green in colour than strain 10. Strain 213 produces conidia very abundantly on Czapek-Dox agar and shows a slight tendency to produce tufts. None of these strains possess the coconut odour characteristic of some strains of *T. viride*, but all produce a bright yellow pigment rapidly and abundantly on Czapek-Dox agar.

This production of pigment appears to bear some relation to viridin production. Weindling (1934) has described three series into which strains of *T. lignorum* (= *T. viride*) fall, viz.

O series, having a characteristic coconut-like odour, producing no pigment.

P series, having no odour, producing a bright yellow pigment on certain media.

Q series, having no odour, producing no pigment.

Bisby (1939) has not discussed this classification of *Trichoderma*, but his observations make it extremely doubtful whether the presence or absence of the coconut odour, distinguishing *O series* from *Q series*, can be considered to be of much significance. However, it is worthy of note that the three strains used in this investigation, and two other viridin-producing strains encountered, all fall into Weindling's *P series*, and the two strains producing gliotoxin described elsewhere (Brian & Hemming, 1945) and two other gliotoxin-producing strains since isolated, all fall into the *Q* or *O series*. Weindling's earlier work (1932, 1934) was all done with a strain of the *P series*,

though the actual isolation of gliotoxin (Weindling & Emerson, 1936) was probably from a strain of the *Q series*. He records that culture filtrates from strains of the *P series* were less stable, and consequently more difficult to handle, than culture filtrates from strains of the *Q series*; consideration of the properties of viridin described later in this paper suggests that his *P series* strains were viridin-producers.

Factors influencing the development of fungistatic activity in culture filtrates

Experimental methods. The cultural methods and methods of biological assay used in this investigation were similar to those previously described (Brian & Hemming, 1945) for production of gliotoxin. Large quantities of conidia of *T. viride* have been needed, and the most satisfactory method found of producing these was on Czapek-Dox agar in flat medicine bottles. With one strain at least it was found that production of conidia was negligible if the medicine bottles were stacked so that the agar surface was horizontal, but that production was vigorous if the bottles were stacked with the agar surface vertical. It appears that the presence of a thin film of precipitated moisture on the agar surface can prevent abundant formation of aerial mycelium and conidiophores.

Depth of medium. In Fig. 1 data are presented graphically showing the fungistatic activity of culture filtrates from three strains of *T. viride*, growing on Weindling medium (see Brian & Hemming, 1945) of three different depths. The culture vessels used in this case were round glass vessels 17·5 cm. in diameter, of the type recently described by Clayton *et al.* (1944). Considering first the results on the 0·5 cm. depth of medium, it will be seen that there is a distinct peak of fungistatic activity between the 6th and 9th days of incubation, followed by a fall in activity. The peak is most pronounced with strain 213, less so with strain 10 and least with strain 214. Considering next the greater depths of medium, it will be seen that increasing the depth of medium delays the attainment of peak activity and lowers the height of the peak; this result might well be expected from the greater volume of medium through which the active substance has to diffuse, and the greater ratio of volume of medium to area of mycelial felt. This would not adequately explain the fact that the total activity produced (i.e. activity in B.A. units per ml. × volume of medium) is noticeably less, particularly with strains 10 and 214, in the 2 cm. depth of medium as compared with the 0·5 cm. depth. This is believed to be due to mechanical instability of the mycelial felt in the deeper media. Strains 10 and 214 produce a slimy, non-sporulating, semi-submerged mycelial felt which in the deeper media tends to sink and doubtless then suffers from a degree of oxygen deficiency. Strain 213, on the other hand, produces abundant aerial mycelium, sporulates freely, and is able to anchor itself to a considerable degree to the sides of the culture vessel. A result of this is that the superiority of strain 213 over the other two is most marked on large culture vessels with relatively great depths of medium, and in smaller culture vessels, such as 100 ml. flasks, the superiority is not so marked.

There is, then, an optimum depth of medium, varying with the strain of mould used, dependent largely on the morphology of the mycelial felt produced.

Table 1. *Fungistatic activity* (B.A. *units/ml.*) *of culture filtrates from three strains of* Trichoderma viride

Strain	Medium	Days growth at 25° C. 4	6	8	10	13
No. 10	Weindling	192	768	3062	1536	2048
	Czapek-Dox	128	384	1024	1024	768
	Raulin-Thom	384	1024	1536	1024	768
No. 213	Weindling	512	1024	1536	2048	1536
	Czapek-Dox	32	128	192	384	384
	Raulin-Thom	1024	2048	16384	8192	3062
No. 214	Weindling	128	384	384	1024	192
	Czapek-Dox	128	32	512	512	128
	Raulin-Thom	192	512	1024	384	512

Composition of medium. The development of fungistatic activity in culture filtrates of three strains of *T. viride* on three standard synthetic media is shown in Table 1. The Czapek-Dox and Raulin-Thom media were made up to the formulae of Raistrick (1931). This experiment was carried out on 30 ml. lots of medium in 100 ml. conical flasks. It will be seen that, in general, Czapek-Dox is inferior to either Raulin-Thom or Weindling media, this being specially marked with strain 213. Reference to Fig. 2 which shows the changes in *p*H of the medium during this experiment (data for Czapek-Dox and Raulin-Thom only are shown as the results on Weindling

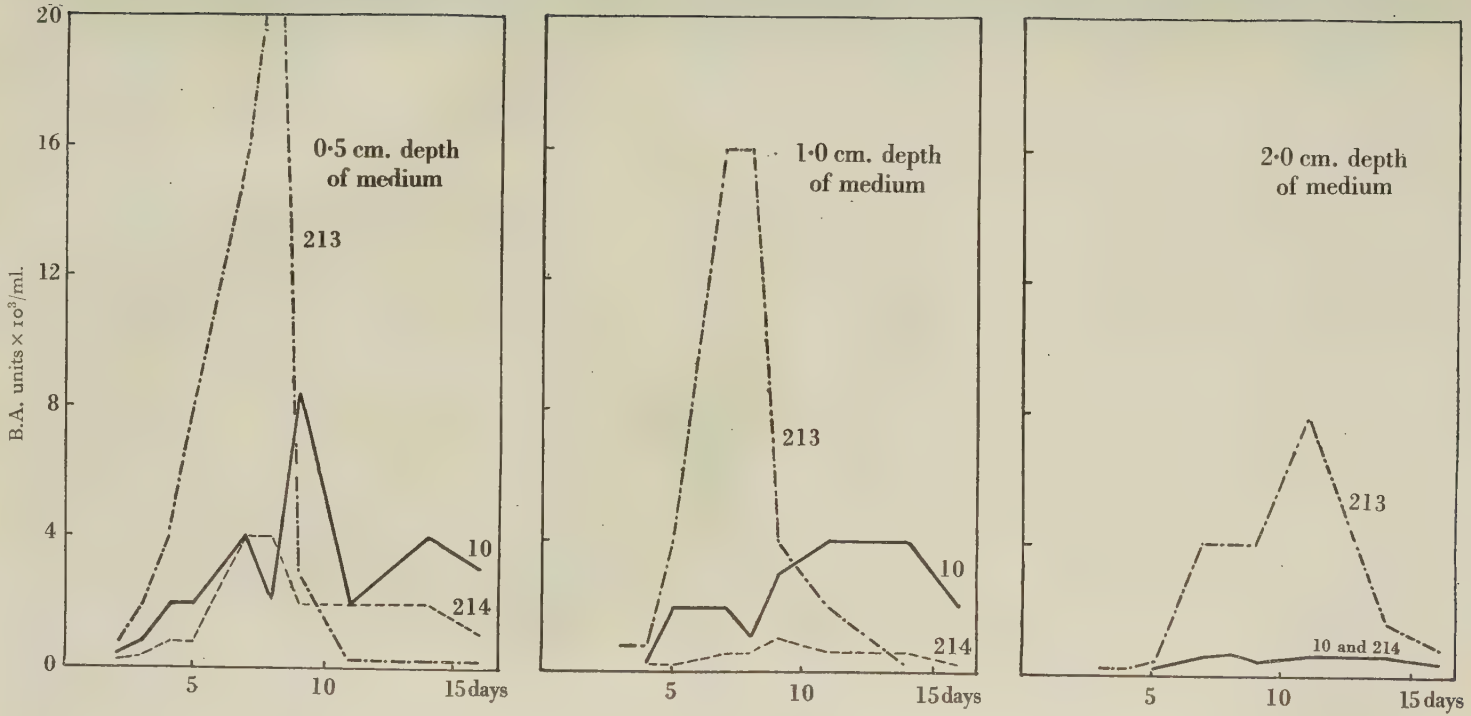

Fig. 1. Development of fungistatic activity in cultures of three strains of *T. viride* on three depths of Weindling medium.

were essentially the same as those on Raulin-Thom), reveals considerable differences between the media in this respect. On Raulin-Thom, the *p*H of the medium with all three strains falls slightly and then remains constant. On Czapek-Dox, there is a considerable rise in *p*H, usually followed by a fall. In the case of strain 213 on Czapek-Dox, the *p*H rises to as much as 7·2; under these conditions, as will be shown later, viridin is exceedingly unstable. It is believed that the unsuitability of Czapek-Dox medium is not due to sodium nitrate being an intrinsically unsuitable nitrogen source (this is the main distinction from the other two media, where the nitrogen is presented as ammonium salts), but that selective absorption of the nitrate ion is liable to lead to a rise in *p*H to a level where viridin is unstable. Further evidence of this may be seen in Table 2 and Fig. 3 where results are given of a similar experiment on media based on the Weindling formula but containing equivalent amounts of nitrogen in various forms. In this experiment sodium nitrate, and to an even greater extent calcium nitrate, have given good results. It will be seen from Fig. 3 that in this case also, culture filtrates containing $NaNO_3$ showed a marked rise in *p*H, but did not reach a level where destruction of viridin would be rapid. Calcium

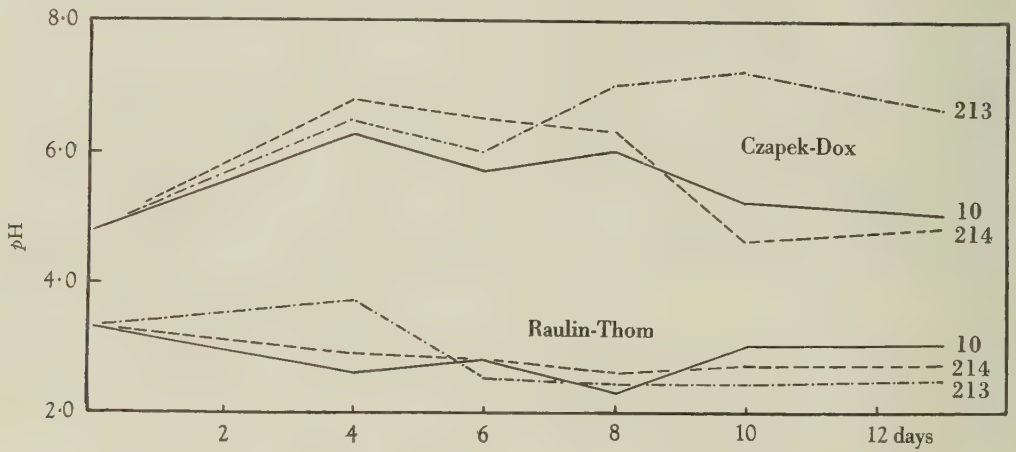

Fig. 2. Drift of *p*H of cultures of three strains of *T. viride* on Czapek-Dox and Raulin-Thom media.

TABLE 2. *Fungistatic activity* (B.A. *units/ml.*) *of culture filtrates from three strains of* Trichoderma viride *on Weindling media with equivalent amounts of nitrogen presented in various forms*

Strain	Nitrogen source	Days' growth at 25° C. 4	6	8	10
No. 10	(1) Amm. tartrate	384	4096	1024	1024
	(2) NH_4NO_3	512	1024	384	1024
	(3) $(NH_4)_2SO_4$	1024	2048	1024	768
	(4) $NaNO_3$	768	2048	8192	2048
	(5) $Ca(NO_3)_2$	512	8192	2048	768
	(6) Peptone	512	4096	1536	3062
No. 213	(1) Amm. tartrate	128	3062	3062	768
	(2) NH_4NO_3	768	2048	3062	3062
	(3) $(NH_4)_2SO_4$	128	384	128	8
	(4) $NaNO_3$	512	4096	2048	2048
	(5) $Ca(NO_3)_2$	384	8192	4096	3062
	(6) Peptone	192	2048	1536	768
No. 214	(1) Amm. tartrate	512	768	384	256
	(2) NH_4NO_3	256	4096	1024	1024
	(3) $(NH_4)_2SO_4$	1024	1024	512	512
	(4) $NaNO_3$	256	2048	1536	1536
	(5) $Ca(NO_3)_2$	768	8192	1024	1024
	(6) Peptone	384	4096	768	768

nitrate showed a less marked rise than sodium nitrate, doubtless because of the less marked basicity of Ca″ as compared with Na′, and this was probably associated with the higher fungistatic activity of culture filtrates. All other nitrogen sources tested show the same slight fall in *p*H. It is noticeable that ammonium sulphate was very unsuitable as a nitrogen source for strain 213; no explanation can be offered for this.

Strain of mould. The results so far presented indicate very clearly the importance of strain variations in connexion with studies of antibiotic metabolic products of moulds. It has been shown that the three strains of *T. viride* chosen for study vary considerably in their reaction to depth of medium and to the composition of the medium. This by no means exhausts the possibilities of strain variation in *T. viride*; it will be remembered that other strains

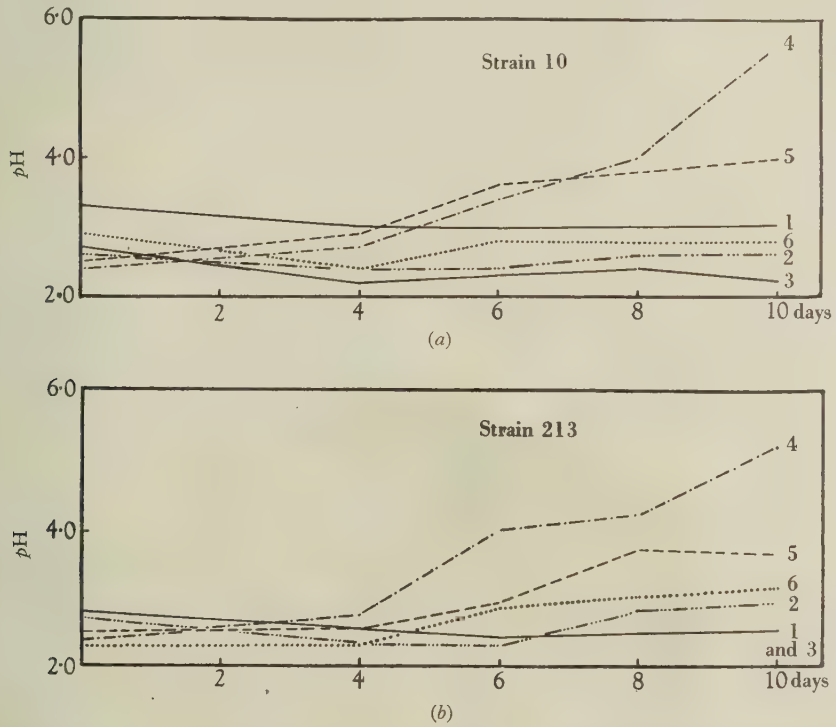

Fig. 3. Effect of different nitrogen sources on drift of *p*H in cultures of *T. viride*: (*a*) strain 10, (*b*) strain 213. (Media are numbered as in Table 2.)

A wide variety of carbon sources can be used for growth of these strains of *T. viride* and many of these are effective in producing highly fungistatic culture filtrates (see Table 3). It is noticeable, however, that the requirements of the different strains of the mould examined seem to vary somewhat. For example, maltose seems somewhat unsuitable for strains 10 and 213, but suitable for strain 214, or lactose is effective for strains 10 and 214 but not for strain 213. The markedly low fungistatic activity on media containing sodium citrate or sodium tartrate as carbon sources is associated with very poor growth; growth was good with all other carbon sources.

produce a totally different antibiotic, gliotoxin, and many strains examined in the course of our studies on viridin and gliotoxin have shown no evidence of production of any antibiotic substance.

Extraction of viridin from culture filtrates

Viridin can be extracted from the culture filtrates by the same method as that adopted for extraction of gliotoxin (Brian & Hemming, 1945). The culture filtrate is extracted with two lots of one-tenth its volume of chloroform and the chloroform evaporated to dryness under reduced pressure. An orange coloured gum with crystals embedded in it is usually

obtained. If this material is dissolved in a little hot ethyl alcohol yellowish white crystals are produced on cooling; if these are recrystallized from ethyl alcohol pure viridin can be obtained in the form of colourless rod-like prisms.

The viridin required for investigation of its chemical and biological properties has been prepared from cultures in flat earthenware vessels of the type described by Abraham *et al.* (1941). Most experience has been obtained with strain 10. With this strain optimum production is obtained using Weindling medium (*p*H 3·5) in 250 ml. quantities; this gives a very shallow depth of medium and extraction is best carried out after 4 days incubation at 25° C. Under these conditions the yield of viridin is of the order of 15 mg./l. of culture filtrate. Higher assay values are sometimes obtained if incubation is allowed to proceed for 5 or 6 days, but the proportion of coloured gummy material to viridin is increased and difficulty is experienced in purifying the viridin. Using the 4-day incubation period it has been found possible to reduce the glucose content of the medium to 1·5% without loss of yield.

Using strain 213, yields of 45 mg./l. have been obtained, the optimum medium in this case being Raulin-Thom. This strain tends to produce more gummy material than strain 10. The fact that it sporulates under these conditions is also a disadvantage as the presence of spores in the culture medium tends to increase the stability of the chloroform emulsion produced during the process of extraction. It has not been possible to use the Weindling-calcium nitrate medium, which the data in Table 2 suggest should be suitable, since in these conditions of culture the *p*H of the culture filtrate occasionally rises to too high a value and no viridin is obtained.

TABLE 3. *Fungistatic activity* (B.A. *units/ml.*) *of culture filtrates from strains of* Trichoderma viride *on Weindling medium with* 2·5% *of various carbon sources*

Strain	Carbon source	Days' growth at 25° C. 4	6	8
No. 10	Dextrose	1024	1024	768
	Glucose (crude)	2048	2048	1024
	Sucrose	512	2048	1024
	Maltose	128	512	64
	Lactose	1024	2048	3062
	Galactose	1024	3062	8192
	Starch	384	384	1024
	Glycerol	768	1536	2048
	Na citrate	—	—	—
	Na tartrate	2	—	—
No. 213	Dextrose	1024	1024	2048
	Glucose (crude)	1024	8192	4096
	Sucrose	384	1024	2048
	Maltose	256	256	512
	Lactose	256	512	768
	Galactose	384	768	1024
	Starch	256	512	1536
	Glycerol	512	512	512
	Na citrate	—	—	—
	Na tartrate	2	16	—
No. 214	Dextrose	192	512	1024
	Glucose (crude)	512	1536	1536
	Sucrose	512	768	384
	Maltose	384	4096	2048
	Lactose	384	3062	3062
	Galactose	384	1024	1024
	Starch	256	768	512
	Glycerol	256	384	2048
	Na citrate	—	—	—
	Na tartrate	4	2	8

PHYSICAL AND CHEMICAL PROPERTIES OF VIRIDIN

Viridin contains no nitrogen, sulphur, or halogens and gives no ash on combustion. It crystallizes from ethanol in colourless, long, rod-like prisms; from methanol in thin plates; and from benzene in short prisms. Whichever of these solvents is used for the purification the analysis agrees with the formula $C_{20}H_{16}O_6$. (Found: C, 68·3, 68·3, 68·0, 68·4, 66·7 and 67·8%; H, 4·8, 4·8, 4·5, 4·8, 5·1 and 4·8%; OCH_3, 8·9, 6·2, 6·6, and 8·8%; mol.wt. (depression of the freezing-point of bromoform, 331.) $C_{20}H_{16}O_6$ requires: C, 68·2%; H, 4·6%; one OCH_3, 8·8%; mol.wt. 352.

Viridin decomposes without melting at 217–223° C.; is soluble in chloroform (a saturated solution in 100 g. of chloroform at room temperature contains 2 g.) and bromoform, is sparingly soluble in carbon disulphide and carbon tetrachloride, is almost insoluble in ether and camphor, gives no colour with alcoholic ferric chloride, and is optically active ($[\alpha]_D^{19}$ for a 1% solution in chloroform is 222°).

Zerewitinoff determinations indicate either one or two atoms of active hydrogen per molecule (Found: H, 0·5, 0·5, 0·4, 0·2%. One H requires 0·3%; two H's require 0·6%), but no nitrogen is evolved when viridin is treated with diazomethane.

Estimations for $CH_3(C)$ groups were in good agreement with one such group. (Found: $CH_3(C)$, 3·4, 3·6, 4·2 and 5·2%. Required 4·3%.) Acetyl determinations carried out by steam distillation after alkaline hydrolysis showed that over two equivalents of volatile acids (2·4 and 2·3) were formed during saponification.

Viridin appears to combine with hydroxylamine and phenylhydrazine but well-defined compounds have not been isolated. However, viridin (0·1 g.) and 2 : 4-dinitrophenylhydrazine (0·16 g.) in glacial acetic acid (40 ml.) deposited a brown microcrystalline powder. After 24 hr., the solid was filtered off and recrystallized from glacial acetic acid (2½ ml.).

(Found: C, 54·8, 52·6 and 51·8%; H, 4·0, 4·0 and 3·6%; N, 11·9, 14·0 and 13·9%; OCH_3, 3·8%. $C_{32}H_{28}O_{14}N_8$ requires: C, 51·3%; H, 3·8%; N, 15·0%; one OCH_3 4·1%.)

$$2C_6H_6N_4O_4 + C_{20}H_{16}O_6 = C_{32}H_{28}O_{14}N_8.$$

Two molecules of 2 : 4-dinitrophenylhydrazine have associated with every molecule of viridin without the elimination of water and a simple dinitrophenylhydrazone has not been formed.

Alcoholic solutions of viridin are reduced by hydrogen in the presence of Raney nickel and in three experiments a colourless gummy product was obtained. A fourth reduction under the same experimental conditions gave a product consisting almost entirely of two compounds which were both isolated. Viridin (1 g.) was catalytically reduced by hydrogen at 50 atm. in the presence of Raney nickel at 100° C. in alcohol (300 ml.) for 5 hr. When cold, the alcoholic solution was filtered to remove the catalyst. The filtrate was pale yellow but rapidly turned a deep violet colour and soon resembled a solution of permanganate. The alcoholic solution was concentrated by evaporation under reduced pressure and a colourless crystalline material separated. This was filtered off and recrystallized twice from hot ethanol in which solvent it was only sparingly soluble. (Found: C, 71·7%; H, 7·6%; OCH_3 nil. $C_{19}H_{24}O_4$ requires: C, 72·1%; H, 7·65%.) This substance was insoluble in camphor.

After removal of the colourless crystalline material the alcoholic solution was evaporated under reduced pressure. A sticky product remained which solidified when stirred with ether. The violet solid so formed was filtered off. The crude product was very soluble in methanol, ethanol, chloroform and 1 : 4-dioxane, and insoluble in ether. It was slightly soluble in water, but the aqueous solutions did not crystallize. It was crystallized from hot benzene in which it was only sparingly soluble, by standing the solution for a week. The violet microcrystalline powder was then filtered off and dried *in vacuo* at 100° C. (Found: C, 66·9, 67·2%; H, 6·6, 6·4%; OCH_3, 7·0, 7·1%; CH_3—attached to carbon, 4·2, 4·4%. $C_{20}H_{22}O_6$ requires: C, 67·1%; H, 6·2%; one OCH_3, 8·7%; one CH_3, 4·2%.) The molecular weight could not be determined by Rast's method since the mixture of the substance with camphor was so deeply coloured that no melting-point could be ascertained. The maximum absorption is at 577 mμ for dilute solutions in chloroform.

The violet compound is surprisingly stable. An alcoholic solution was not affected by cold dilute sulphuric acid, dilute hydrogen peroxide or ferric chloride. Alkalis, picric acid, and acetic anhydride containing traces of sulphuric acid all destroyed the colour. Reducing agents will decolorize the compound. With hydrogen and a palladium-strontium carbonate catalyst the colour disappeared and returned when the liquid was agitated in air. Further quantities of hydrogen again decolorized the liquid and by successive treatments with air and hydrogen the colour could apparently be produced and removed as often as desired. Solutions of hydrogen sulphide also decolorized the compound. The colour returned on shaking with air whilst hydrogen sulphide was still present. Hydrogen sulphide solutions in water are generally considered to be fairly stable. In the presence of the violet reduced viridin, however, such solutions are at once oxidized by air and sulphur is deposited. The violet compound appears in this reaction to fulfil all that is required of a catalyst. It accelerates a reaction (a relatively small amount producing a large effect in the reacting mixture) and is itself unchanged at the end. A number of organic compounds such as methylene blue can act as oxygen carriers in linked enzymatic reactions, but catalysis by the violet compound is unusual in two respects. First, the compound itself is the catalyst and not a mere link in an enzymic process and secondly, this organic catalyst is not ionic either in the oxidized or in the reduced state.

The formation of the two hydrogenated compounds would be explained by the equation below:

$$\begin{array}{l} 7H_2 \nearrow \quad C_{19}H_{24}O_4 + CH_3OH + H_2O \\ C_{20}H_{16}O_6 \\ \qquad\quad 4H_2 \qquad\quad -H_2 \\ \qquad\quad \searrow \quad C_{20}H_{24}O_6 \rightleftharpoons C_{20}H_{22}O_6 \\ \qquad\qquad\qquad\quad +H_2 \end{array}$$

Neither the compound $C_{19}H_{24}O_4$ nor the compound $C_{20}H_{22}O_6$ had any fungistatic activity.

The exact nature of the six oxygen atoms in viridin is not yet clear. One oxygen atom is present in a methoxyl group, and since this group is removed during the catalytic reduction of viridin to a compound of apparent formula $C_{19}H_{24}O_4$ the methoxyl group may be part of a carbomethoxy group. Alkaline hydrolysis gives rise to over two equivalents of volatile acid per molecule of $C_{20}H_{16}O_6$ and since a $CH_3(C)$ group has been found, one of these acids may be acetic acid. The remaining two oxygen atoms may be present as hydroxyl groups because Zerewitinoff determinations show one or two atoms of active hydrogen per molecule, and as the addition of diazomethane yields no nitrogen the hydroxyl groups may be of an alcoholic nature. The tentative formula for viridin could, therefore, be written as follows:

$$\begin{array}{l} \qquad\quad COOCH_3 \\ \qquad\quad / \\ C_{16}H_8\text{—}OCOCH_3 \\ \qquad\quad \backslash \\ \qquad\quad (OH)_2 \end{array}$$

The colour of the compound $C_{20}H_{22}O_6$ obtained by hydrogenating viridin is interesting in that it may indicate the presence of a conjugated system, a surmise supported by the low hydrogen content of the radical $C_{16}H_8$.

At this stage the experimental evidence is too meagre to suggest a structural formula but now that larger quantities of viridin are available more extended experimental work will be possible.

Biological activity of viridin

Fungistatic and fungicidal activity. The lowest concentration of viridin inhibiting germination of a number of fungi is given in Table 4. All these figures were determined in a germination medium at *p*H 3·5, the composition of which has been given elsewhere (Brian & Hemming, 1945).

Table 4. *Toxicity of viridin to spores of various fungi*

Organism	Least conc. (μg./ml.) inhibiting germination: Viridin	$HgCl_2$
Aspergillus niger	3·1	0·25
Botrytis allii	0·006	0·25
Cephalosporium sp.	0·8	0·25
Cladosporium herbarum	0·2	0·5
Colletotrichum lini	0·003	0·25
Fusarium caeruleum	0·003	0·25
F. culmorum	0·2	0·125
Penicillium digitatum	0·2	2·5
P. expansum	6·25	1·0
P. notatum	0·2	0·5
Stachybotrys atra	6·25	0·5
Stemphylium sp.	0·2	0·25
Trichoderma viride (strain 10)	>50·0	0·25
T. viride (strain 12)	50·0	0·25
Trichothecium roseum	0·05	0·25

Viridin prevents germination of the spores of such fungi as *Botrytis allii, Colletotrichum lini* and *Fusarium caeruleum* at remarkably low concentrations. Values of the least inhibiting concentration of viridin to *Botrytis allii* have, in a large number of experiments, varied between 0·001 and 0·05 μg./ml. with a mean value about 0·01 μg./ml. Results presented elsewhere (Brian & McGowan, 1945) show that this is an activity equal to, or exceeding, that of such active fungicides as di(ethyl-mercuri) hydrogen phosphate. Other fungi are more resistant; concentrations of the order of 6μg./ml. are required to inhibit germination of the spores of *Penicillium expansum* or *Stachybotrys atra.* Viridin is rather less toxic than mercuric chloride to these fungi. Both strains of *Trichoderma viride* tested show a remarkable tolerance of viridin, a concentration more than 5000 times that required to inhibit germination of *Botrytis allii* having no effect in the case of strain 10. Viridin is, therefore, highly fungistatic to certain fungi, but shows a pronounced degree of specificity.

Much higher concentrations are required to be rapidly fungicidal. Spores of *Botrytis allii* and *Stemphylium* sp. were suspended in aqueous solutions of viridin, buffered at *p*H 3·5, of concentrations varying from 0·1 to 50·0 μg./ml. for periods of 25 min. and 2 hr. The spores were then centrifuged out, washed twice in germination medium, and then placed in drops of germination medium in moist chambers and incubated at 25° C. *Botrytis allii* conidia were killed by concentrations of viridin of 50 and 10 μg./ml. in 25 min. or 2 hr., but not by 1 μg./ml. in 2 hr. or less. Conidia of *Stemphylium* sp. were killed by 25 min. exposure, or longer, to 50 μg./ml. of viridin but not by 2 hr. exposure to 10 μg./ml. Thus to kill conidia of *Botrytis allii* in 2 hr. a concentration is required 1000 times greater than that which will inhibit germination if present in the germination medium, and for *Stemphylium* sp. a concentration 200 times greater. Thus, although viridin has pronounced fungicidal powers, it is its fungistatic activity which is most striking.

Bacteriostatic and bactericidal activity. Using the normal serial dilution technique in broth, viridin does not inhibit growth of *Staphylococcus aureus, Escherichia coli* or *Salmonella typhi* at concentrations as high as 100 μg./ml. This is not conclusive proof of low bacteriostatic activity, as it is shown later in this paper that viridin rapidly loses fungistatic activity at or near neutrality, the usual reaction of bacteriological culture media.

It can be shown that viridin is not an active bactericide. Cells of *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* were suspended in citrate-phosphate buffers, of *p*H ranging from 3·7 to 7·3, containing 0, 10 and 100 μg./ml. viridin, at 20° C. Loopfuls were taken out every 6 min. for 1 hr. and streaked on to nutrient agar plates. The buffers alone were not bactericidal. Viridin at 100 μg./ml. had no effect at any *p*H, save that the 60 min. exposure to 100 μg./ml. viridin at *p*H 3·7 showed some slight kill of *Staphylococcus.*

It may be concluded from this evidence that viridin has negligible antibacterial properties. Viridin is thus distinguished from all previously described mould antibiotics in that it is fungistatic but not bacteriostatic. Such substances as clavacin (also known as patulin or claviformin) (Waksman & Bugie, 1943; Anslow *et al.* 1943), gliotoxin (Brian & Hemming, 1945) and fumigacin (Waksman & Bugie, 1943), which have been described as possessing fungistatic properties, are also bacteriostatic.

Stability of aqueous solutions of viridin in relation to *p*H

Aqueous solutions of viridin are unstable, the fungistatic activity falling rapidly. This instability is

related to *p*H, as examination of the figures in Table 5 will show. The viridin was dissolved in a little ethyl alcohol, and portions of this were added to sterile citric acid-phosphate buffer so that the final concentration of alcohol was 2% and of viridin 50 μg./ml. At 25° C. all activity is lost within 5 hr. at *p*H 7·6 or above; at *p*H 3·8 there is also a considerable fall in activity, but appreciable activity can still be detected after 7 days; at *p*H 2·9 aqueous viridin solutions are relatively stable. The solutions turn yellow as activity is lost. Weindling (1932, 1934) has shown that *Trichoderma* is most actively antagonistic to other fungi in acid soils; this can be very definitely correlated with the instability of gliotoxin in aqueous solutions except at low *p*H (Brian & Hemming, 1945) and the still greater instability of viridin. The probable rapid disappearance of viridin in many soils does not preclude it from having considerable biological influence. When it is recalled that culture media after 8 days' growth of a viridin-producing strain of *T. viride* may have a fungistatic activity of 16,384 B.A. units per ml., it can be imagined that, even allowing for decomposition of viridin, the neighbourhood of a viridin-producing *Trichoderma* hypha in the soil may be markedly inhibitory to other fungi.

Table 5. *Effect of p*H *on fungistatic activity of aqueous solutions of viridin* (50 μg./ml.) *maintained at* 25° C.

Period of storage	Activity in B.A. units/ml.				
	*p*H 2·9	*p*H 3·8	*p*H 5·8	*p*H 7·6	*p*H 8·4
15 min.	3072	3072	2048	512	—
5 hr.	2048	2048	512	4	—
1 day	1024	1024	4	—	—
2 days	1024	1024	—	—	—
3 days	1024	512	—	—	—
6 days	1024	512	—	—	—
10 days	1024	256	—	—	—

Inactivation of viridin by other substances of metabolic importance

Waksman & Bugie (1943) have shown that the antifungal activities of clavacin and actinomycin can be partially overcome by such substances as peptone. Similarly, if viridin is kept in solution at *p*H 3·5, loss of activity is much accelerated in the presence of such substances as peptone, Marmite or Difco Yeast-extract. These substances are complex mixtures, and study of the effect of single compounds likely to be present is more likely to yield results of value. In this connexion, Cavallito & Bailey (1944) claim that a number of mould anti-biotics, including gliotoxin, patulin and penicillic acid, are inactivated by cysteine. The action of a number of growth factors, amino-acids, etc. on viridin has been examined, and, as will be seen from the data in Table 6, a number of these do have an inactivating effect on viridin.

With sterile precautions, solutions were made by mixing 0·5 ml. alcoholic viridin, 8·5 ml. *p*H 3·5 buffer (0·2 *M* phosphate/0·1 *M* citric acid) and 1·0 ml. aqueous solution of the potential inactivating agent, so that the final concentration of viridin was 2 μg./ml. and of the potential inactivating agent 100 μg./ml. These solutions were assayed immediately and after 6 days' storage at 25° C. Results are presented in Table 6. Only *dl*-alanine, choline chloride, glycine and inositol have any immediate deactivating effect on viridin under these conditions. On the other hand, *p*-aminobenzoic acid, thioglycollic acid and l-tryptophane, though more slow in action, have a more pronounced final action. No particular significance can at present be attached to these results, though they do indicate that the inactivating effect of such substances as peptone may be due to several of its constituents.

Table 6. *Activity of viridin in presence of excess of various growth factors and amino-acids*

Substance added	Fungicidal activity in B.A. units/ml.	
	Immediate	After 6 days
—	64	32
dl-Alanine	16	16
Aneurin hydrochloride	32	32
p-Aminobenzoic acid	32	—
Choline chloride	16	16
Cysteine hydrochloride	64	32
l-Cystine	64	32
Glutamic acid	64	32
Glycine	16	16
Inositol	16	8
Nicotinic acid	32	16
β-phenyl alanine	128	32
Pyridoxin	64	32
Riboflavin	64	32
Thioglycollic acid	32	—
l-Tryptophane	64	8
l-Tyrosine	64	32

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Diseases of the gladiolus

III. *Botrytis* rot of corms and its control

By Lilian E. Hawker, *Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London*

The probability that the three types of corm rot of gladioli, associated with *Botrytis* spp., described by Moore (1939) are phases of the same disease is discussed.

A large number of isolations all yielded similar strains of *B. cinerea*. *B. gladioli* Kleb. was not observed. Inoculation experiments with the isolated strain of *B. cinerea*, with pieces of diseased tissue or with sclerotia taken from diseased corms, gave positive results under certain conditions. Wounding, particularly at the base of the old shoot or at the base of the corm, followed by cool, moist storage increased the proportion of positive results.

Control measures were of five types: (1) Measures to reduce the amount of active or dormant infection of the corms when lifted. Avoidance of heavy wet soil, ground in which gladioli had previously grown and shaded sites reduced the subsequent loss in storage. Relatively early lifting (during the first half of October) was beneficial. Rogueing of diseased plants during the summer and dusting of foliage with pentachloronitrobenzene dust (p.c.n.) gave some improvement, but further experiments are desirable. (2) Measures to reduce the development of the disease between lifting and storage. Early removal of adhering soil, cormels, roots, parent corms and old shoot and leaf bases (husks) gave good control. Rapid drying in warm, dry conditions was also beneficial, but washing the corms to remove soil was ineffective. Dusting with p.c.n. immediately after lifting and before cleaning gave a slight reduction in losses in two small-scale experiments. (3) Pre-storage fungicidal treatment of corms. Dusting with p.c.n. before storage gave good control, but almost complete control was obtained by steeping in a 0·1 % solution of mercuric chloride for 20 min. to 3 hr. This treatment has the advantage that it also controls hard rot (*Septoria gladioli* Passer.), scab (*Bacterium marginatum* McCull) and to a lesser extent dry rot

(*Sclerotinia gladioli* Drayt.), but is liable to damage the corms. The safety limits are being worked out. (4) Conditions of storage. Corms should be stored in shallow trays in a dry, well-ventilated shed, protected from frost. (5) Fungicidal treatment at planting time to protect the corm from soil infection. Mercuric chloride as a corm steep at this time gave only partial control, probably owing to rapid removal of the fungicide by leaching. Dusting with p.c.n. gave good results, not only in the protection of the old corm but in preventing infection of the new ones and the beneficial effect extended into the subsequent storage period. Soil treatment was not effective.

Probable relation between types of disease associated with *Botrytis*

Moore (1939) described three forms of rotting of gladiolus corms associated with species of *Botrytis*. Losses in England were severe from 1936 onwards, but earlier records of the disease were infrequent.

Gladioli have been cultivated at the Biological Field Station of the Imperial College of Science at Slough since 1938. Losses in storage from *Botrytis* rot have occurred each year. These were negligible following the abnormally hot dry summer of 1940, but have been considerable in all other years and severe in those years when late summer and autumn were wet. The first type of rot described by Moore in which 'sunken rounded lesions, straw-coloured in the centre but deep brown or reddish brown at the margin, occur on the corms' and in which 'below the tightly stretched and often cracked skin there is usually a cavity' has been seldom seen. The other two types, namely, a type 'in which the whole corm becomes affected with a spongy rot' and the well-known core-rot have been very frequent. Observations confirmed Moore's suggestion that core-rot may develop into spongy rot. The latter may also develop from infection entering, usually through wounds, at any point of the corm surface. Thus among 346 corms of various varieties rotting in storage during the winter of 1939–40 and examined in March, ninety-six were completely rotten (Moore's spongy type), 197 showed core-rot spreading along the vascular strands and into the ground tissue of the corm, many of them being almost completely rotten and approaching the spongy type, forty-seven showed core-rot progressing downwards from a point of infection at the scar left by the old shoot, twenty-three showed similar rot progressing upwards from the basal plate, and fifty-one showed rot starting from the surface at some point other than the old shoot or base. As in previous work (Hawker, 1944; Hawker *et al.* 1944), the husks or old leaf bases were removed from the corms before storage. Usually the old stem base could be lifted out leaving a clean depressed scar. When this could not be done the corms often developed core-rot later. This occurred particularly frequently with the varieties Yvonne and Picardy.

Corms which were wound-inoculated with *Botrytis* and subsequently stored at rather high temperatures (20–25° C.) showed a walling-off of the infected areas by the formation of callus, and it is suggested that the first type of rot described by Moore, which he states is more frequent in Canada than in England, may be due to such an effect of relatively high temperature tending to localize the infection. The writer has only found this type of rot, in naturally infected corms, after hot summers. Thus Moore's three types of rot may be different forms of the same disease.

Losses also occurred in the ground during the growing period but were usually less serious than those during storage. Failure of shoot emergence was often due to the development of core-rot in the parent corm in the first few weeks after planting. Such corms probably carried a dormant form of the fungus when planted. After shoot emergence, premature yellowing of the foliage occurred as a result of *Botrytis* attack on the parent corm shoots or young corms. *Botrytis* was isolated from such plants. Rotting of young shoots at or below ground-level and of young corms may be due to mycelium (probably developing from sclerotia) in the soil. These conclusions are supported by the results of fungicidal treatment described below in which surface sterilization of the corms with mercuric chloride reduced the loss from the old corms rotting after planting but did not prevent infection of new corms during the growing season. Moore describes spotting of foliage and petals and rotting of shoots by *Botrytis*. It is likely that spores formed on the rotting shoots may be washed down by rain and may provide an alternative means by which the young corms become contaminated. These may either already be rotten at lifting time or may develop the disease in storage.

Isolations from diseased corms

Isolations from corms showing these types of rot invariably yielded strains of *Botrytis*. All the isolates were essentially similar in cultural appearance and produced both conidia and sclerotia. The size and shape of the latter varied with the medium. Conidia were spherical to ellipsoidal and within the size range given by Moore, viz. $12–15 \times 9–12\,\mu$. No cylindric-ellipsoidal conidia of the type described by Klebahn (1930) and named *B. gladioli* Kleb. were seen in any of the isolates. The isolate is considered to be a strain of *B. cinerea*. The morphology, physiology and host range of a typical isolate are being examined in

this laboratory by Pieris and compared with those of strains of *Botrytis* from other hosts.

Inoculation experiments

Moore succeeded in obtaining typical core-rot in corms of variety Pfitzer's Triumph inoculated with a strain of *Botrytis* from diseased corms, and stored in moist chambers at 24° C., but obtained negative or inconclusive results with other varieties. Experiments now in progress in this laboratory indicate that, with most varieties, cork formation at temperatures of 20° C. or over is sufficiently rapid to prevent the establishment of the disease in inoculated corms. Burrows (unpublished data) obtained positive results in five out of nine corms (var. Vesuvius) inoculated in May and stored at room temperature.

A hundred corms of various varieties were wound-inoculated with pieces of agar culture of a typical isolate during the winter and were stored in an unheated shed: twenty-one of them developed typical spongy or core-rot. All but one of the controls remained sound.

A number of corms were inoculated in March and planted immediately afterwards. Pieces of agar culture of *Botrytis*, pieces of diseased tissue or sclerotia (taken from diseased corms and surface sterilized by immersion for 1 min. in a 0·1 % solution of mercuric chloride followed by two rinsings in sterile water) were used as inocula. These were placed over wounds at the 'neck', i.e. scar of old shoot, side or base of the corm or were similarly placed without previous wounding. The inocula were protected by a piece of damp cotton-wool. Cotton-wool was

Table 1. *Inoculation experiments*

Variety	No. inoculated	Method of inoculation	Inoculum	No. rotting in soil
Yvonne	5	Wound, side	Agar culture	1
	5	Wound, neck	Agar culture	0
	5	Wound, base	Agar culture	4
	5	Contact, side	Agar culture	0
	5	Contact, neck	Agar culture	1
	5	Contact, base	Agar culture	1
	5	Wound, side	Sclerotia	0
	5	Wound, neck	Sclerotia	1
	5	Wound, base	Sclerotia	1
	5	Contact, side	Sclerotia	0
	5	Contact, neck	Sclerotia	0
	5	Contact, base	Sclerotia	0
	5	Wound, side	Diseased tissue	1
	5	Wound, neck	Diseased tissue	2
	5	Wound, base	Diseased tissue	4
	5	Contact, side	Diseased tissue	0
	5	Contact, neck	Diseased tissue	2
	5	Contact, base	Diseased tissue	1
	15	Wounded	None	0
	15	Unwounded	None	0
Mrs Maclaren	12	Wounded	Spore suspension	7
	12	Unwounded	Spore suspension	6
	12	Wounded	None	4
	12	Unwounded	None	5
Afterglow	10	Wound, neck	Sclerotia	4
	10	Wound, neck	None	3
America	5	Wound, neck	Agar culture	4
	5	Wound, side	Agar culture	3
	5	Wound, neck	None	1
	5	Wound, side	None	2
Lilac Wonder	20	Contact, neck	Sclerotia	8
	20	Unwounded	Sclerotia	7
Vesuvius	5	Wound, neck	Sclerotia	1
	5	Contact, neck	Sclerotia	0
	5	Wound, neck	None	0
	5	Unwounded	None	0
Foch	12	Planted over inoculum	Sclerotia	10
	12	Planted over inoculum	Diseased tissue	6
	12	Untreated	None	0

similarly applied to wounded and unwounded controls. The corms were previously surface sterilized by immersion in 0·1% mercuric chloride and thoroughly washed. In one experiment the corms were dipped in a suspension of *Botrytis* spores before planting, and in another they were planted over sclerotia or minced diseased corms.

The proportion of inoculated corms rotting (Table 1) was usually low but was increased by wounding. Thus 33/82 wound-inoculated and 35/106 contact-inoculated corms rotted. In three experiments in which wound- and contact-inoculation were directly compared, 22/62 wounded and 11/62 unwounded corms rotted. Inoculation at neck or base was usually more effective than similar inoculation at the side. In one experiment the numbers rotting were 6/15, 11/15 and 2/15 for inoculation at neck, base and

losses of corms from these two plots, in the ground and in storage, are recorded in Table 2, which shows that the wet plot favoured the disease.

In one experiment losses among corms grown in wet soil where gladioli had been grown previously were double those suffered in similar soil which was planted with gladioli for the first time. This may have been due to the presence in the soil of an increased amount of inoculum in the form of sclerotia or mycelium (probably encouraged by the unavoidable presence of gladiolus debris from the preceding crop), or to an increased chance of entry via lesions caused by other gladiolus pathogens known to survive in the soil from one growing season to the next.

Thus gladioli should not be planted in wet, shaded sites nor in soil which has previously carried a diseased crop.

TABLE 2. *Losses in stocks of var. Lilac Wonder grown in 'dry' and 'wet' soils*

Soil conditions	No. planted	No. old corms rotting in ground	No. new corms rotted when lifted	No. new corms stored	No. rotting in storage	Disease* index
Wet	400	11	3	472	227	22·75
Dry	400	1	0	484	38	4·05
Wet	—	—	—	480	27	—
Dry	—	—	—	480	3	—
Wet	160	7	1	385	25	13·13
Dry	160	3	1	359	22	7·94
Wet	160	8	Not cleaned until March	380	138	33·75
Dry	160	2	Not cleaned until March	348	115	13·06

* Disease index calculated as in previous paper (Hawker, 1944).

side respectively. Diseased tissue was the most and sclerotia the least effective type of inoculum.

In one experiment in which pieces of sporing agar culture of the *Botrytis* strain were placed at ground level adjacent to the shoot in September, 11 out of 25 corms were rotten when lifted in October compared with 3 out of 25 uninoculated corms.

CONTROL MEASURES

The incidence of the disease in storage and during the growing season suggests several types of control measure.

(1) *Measures designed to reduce the amount of active or dormant infection of the corms when lifted*

(*a*) *Choice of site.* Gladioli have been grown at Slough in a light quick-drying soil exposed to the full effect of sun and wind (dry plot) and in a heavier low-lying soil protected from some of the effects of sun and wind by trees and hedges (wet plot). The

(*b*) *Rogueing of diseased plants.* Plants bearing sclerotia or conidia of *Botrytis*, as described by Moore, are potential foci of infection, and the early removal of all plants showing yellowing of the foliage is an obvious precaution. During work on other gladiolus diseases all corms which failed to develop or which showed premature yellowing of foliage were removed and the cause of failure was determined. Losses in storage from *Botrytis* in these experimental lots were less than in stock lots which had been less carefully rogued.

(*c*) *Fungicidal treatment of foliage.* In two experiments the foliage was dusted with a pentachloronitrobenzene dust (p.c.n.)* which was shown by Brown (1935) and Smieton & Brown (1940) to control the *Botrytis* disease of lettuce. Table 3 shows that slightly reduced losses followed such treatment.

(*d*) *Early lifting.* It has already been shown that, under conditions favourable to the fungus, young

* Marketed under the name of Folosan.

corms may become infected in the ground. In severe cases these may be already rotten when lifted but more often the disease develops in storage. The chance of such infection increases with the time the corms are left in the ground, particularly in wet autumns. Corms of var. Lilac Wonder were lifted at approximately fortnightly intervals from early October to late November 1944. Half of each batch was cleaned 2 weeks after lifting and the remainder were all cleaned on the same day (4 Jan. 1945).

Table 4 shows that early lifting while the soil is comparatively dry, followed by quick drying and cleaning reduced losses to a low level. In the second cleaned corms are put into storage. It is desirable, therefore, to increase the rate of drying by spreading out the corms, by bringing them into a warm, dry room or by other means.

(*a*) *Rate of drying.* Batches of fifty corms var. Lilac Wonder were stored for 1 month after lifting in an unheated shed (cool, moist conditions), a heated greenhouse (warm, moist), an unheated room (cool, dry), and a heated corridor (warm, dry). Batches of 100 corms of var. War were stored in the shed and in the greenhouse. The corms were then cleaned and stored in trays in the bulb shed. Table 5 shows that warm storage for 1 month, even under

TABLE 3. *Effect of foliage dusting on subsequent loss from* Botrytis

Variety	Foliage treatment	Type of soil	No. planted	Total new corms	No. corms rotting in storage
Rose Precose	6 dustings p.c.n., Sept.–Oct.	Wet	80	107	38
	None	Wet	80	102	53
Ave Maria	7 dustings p.c.n., Sept.–Oct.	Dry	50	65	0
	None	Dry	50	52	6

TABLE 4. *Effect of date of lifting on* Botrytis *rot during storage*

Date lifted	Soil conditions when lifted	Date cleaned	Total new corms	Number sound survivors March 1945
9. x. 44	Fairly dry	24. x. 44	89	82
24. x. 44	Wet	7. xi. 44	70	45
7. xi. 44	Wet	21. xi. 44	78	54
21. xi. 44	Very wet	4. xii. 44	81	5
9. x. 44	Fairly dry	4. i. 45	81	22
24. x. 44	Wet	4. i. 45	82	5
7. xi. 44	Wet	4. i. 45	90	7
21. xi. 44	Very wet	4. i. 45	79	1

TABLE 5. *Effect of storage conditions immediately after lifting on subsequent loss from* Botrytis

Variety	Storage conditions for 1 month after lifting	No. stored	No. rotting in March
Lilac Wonder	Cool, moist	50	14
	Warm, moist	50	3
	Cool, dry	50	8
	Warm, dry	50	0
War	Cool, moist	100	16
	Warm, moist	100	2

and third batches, lifted when the soil was moderately wet, losses were considerable in those cleaned a fortnight after lifting and heavy in those in which cleaning was delayed. The last lot, lifted from very wet soil, showed almost complete loss even in those cleaned soon after lifting.

In two small-scale experiments with vars. America and Lilac Wonder, in which corms were lifted at intervals from a light soil in a relatively dry autumn, losses were negligible. Early lifting did not significantly reduce the weight of the new corms.

(2) *Measures to reduce the development of the disease between lifting and storage*

If the fungus is present, either in a dormant or an active form, on the corms when lifted, it is likely to find conditions more favourable for attack before the corms are thoroughly dried than when the dried and the moist conditions of the greenhouse, reduced subsequent losses. This may be due, not only to rapid drying, but also in part to the development of callus over wounds. (A similar effect was noted by Lauritzen & Wright (1934) with storage rot due to *Penicillium gladioli* McCull & Thom.) Cool, dry storage after lifting was better than cool, moist conditions.

Corms of the susceptible varieties Mrs Maclaren and Yvonne were washed immediately after lifting from wet ground to remove adhering soil. No improvement was seen compared with unwashed batches.

(*b*) *Date of cleaning.* The effects of the length of the interval between lifting and cleaning and of the extent to which the cleaning process was carried out were also examined. Hawker (1944) and Hawker *et al.* (1944) found that it was necessary to remove the

old leaf bases (husks) and shoot as well as parent corm, cormels and roots as soon as possible after lifting, in order to determine the degree of infection with hard or dry rots. This was harmless to the corms. In commercial practice, the cleaning process is limited to the removal of the old corm, cormels and roots together with any adhering soil, leaving the old shoot (cut off to within a few inches of its base) and leaf husks attached to the new corm. Neither process can be carried out without injury to the corms until the latter have been dried to a certain extent. Table 6 shows that corms should be cleaned not later than 1 month after lifting and that complete cleaning is better than partial cleaning (i.e. to the extent practised by the growers).

with p.c.n. (Table 7), but losses in the control lots were low, particularly in 1940, probably as a result of the abnormal drought of the preceding summer. Dusting with Brassisan or Ceresan was usually less effective, and these dusts were not used in later experiments. A number of proprietary fungicides recommended for use against *B. tulipae* were tested in the autumns of 1942 and 1943. Two of these compared favourably with p.c.n. but were omitted from the experiments in the autumn of 1944 owing to the greater efficiency of mercuric chloride. Hawker (1944) stated that, in experiments to test the relative efficacy against hard rot of autumn treatment and spring treatment with mercuric chloride or Aretan, the autumn treatment prevented losses from *Botrytis*.

TABLE 6. *Effect of date and extent of cleaning of corms on losses during storage*

Variety	Date lifted	Date cleaned	Degree of cleaning	No. old corms	No. new corms	No. of survivors in March
Salmon Beauty	14. x. 41	30. x. 41	Complete	40	52	43
	14. x. 41	—	Not cleaned	40	54	24
Van Tienhoven	14. x. 41	30. x. 41	Complete	50	96	95
	14. x. 41	—	Not cleaned	50	95	52
Rose Precose	14. x. 41	30. x. 41	Complete	112	215	160
	14. x. 41	30. xi. 41	Complete	112	205	60
Ave Maria	7. x. 42	28. x. 42	Complete	90	90	79
	7. x. 42	28. x. 42	Partial	90	90	27
	7. x. 42	—	Not cleaned	90	90	15
Lilac Wonder	18. x. 44	26. x. 44	Complete	—	50	45
	18. x. 44	7. xi. 44	Complete	—	50	47
	18. x. 44	21. xi. 44	Complete	—	50	38
	18. x. 44	15. xii. 44	Complete	—	50	13
	18. x. 44	4. i. 45	Complete	—	50	9
	18. x. 44	6. ii. 45	Complete	—	50	8
	18. x. 44	—	Not cleaned	—	50	0
	Oct.–Nov. 1944	14 days after lifting	Complete	—	318	186
	Oct.–Nov. 1944	4. i. 45	Complete	—	332	35

(*c*) *Dusting with p.c.n. immediately after lifting.* A light dusting with p.c.n. immediately after lifting reduced subsequent losses in storage from 5/52 to 1/58 with var. Ave Maria and from 4/39 to 1/36 with var. Lilac Wonder. Both these stocks were lifted from relatively dry soil. The treatment would not be practicable with large stocks.

(3) *Pre-storage fungicidal treatment of corms*

Burrows (unpublished data), working in this laboratory, treated 1777 corms, including six different varieties, with two fungicidal dusts (p.c.n. and Brassisan) in the autumn of 1938 and obtained a reduction in losses from *Botrytis* during storage from 23·8% in untreated corms to 2·5 and 7·0% in those treated with p.c.n. and Brassisan respectively.

Experiments by the writer in the autumns of 1939 and 1940 gave a reduction in losses in corms dusted

Consistently good control of *Botrytis* rot in storage has been obtained by steeping the corms in the autumn in 0·1% mercuric chloride for 20 min., 1 or 3 hr. Delay in shoot emergence and in flowering caused by the mercuric chloride treatment was negligible in corms treated in 1942 and 1943, but in those treated in 1944 a number failed to develop roots and either produced no shoot or put out a weak one which soon died. This was probably due to too short an interval between lifting and treatment. Experiments are planned to determine how long this interval should be and the minimum duration of treatment and strength of solution necessary for control of the disease. The use of mercuric chloride or of an appropriate proprietary mercurial fungicide has obvious advantages, since it has been shown to control hard rot (Hawker, 1944), scab (McCulloch, 1924; Hawker, unpublished data) and to a lesser

TABLE 7. *The effect of pre-storage fungicidal treatment on subsequent losses from* Botrytis

Variety	Date of treatment	Corms per treatment	None	P.c.n.	Brassisan	Ceresan	20 min. 0·1% $HgCl_2$	1 hr. 0·1% $HgCl_2$	3 hr. 0·1% $HgCl_2$	15 min. Aretan at 43° C.	Calomel	Shirlan	Tulisan	OB72
Rose Precose	Nov. 1939	25	4	1	1	1	—	—	—	—	—	—	—	—
Lilac Wonder	Nov. 1939	30	6	4	9	6	—	—	—	—	—	—	—	—
Ave Maria	Nov. 1939	80	11	2	4	4	—	—	—	—	—	—	—	—
Six varieties	Nov. 1940	300	5	0	3	—	—	—	—	—	—	—	—	—
Ave Maria	Nov. 1942	25	9	0	—	—	—	—	—	—	—	0	—	—
Prince of Wales	Nov. 1942	40	17	5	—	—	—	—	—	—	—	12	—	—
Copernicus	Nov. 1942	40	12	9	10	—	—	—	—	—	—	9	8	—
Afterglow	Nov. 1942	20	8	1	—	—	—	—	—	—	—	—	—	—
Van Tienhoven	Nov. 1942	90	21	7	—	—	—	—	—	—	—	—	—	—
Ave Maria	Nov. 1942	80	59	45	—	—	—	—	—	—	—	49	—	—
War	Nov. 1942	100	12	—	—	—	—	—	0	1	—	—	—	—
Wolfgang v. Goethe	Nov. 1942	50	18	—	—	—	—	—	3	—	—	—	—	—
Lilac Wonder	Nov. 1943	120	8	5	—	—	0	—	—	—	—	1	0	0
Van Tienhoven	Nov. 1943	40	9	3	—	—	0	—	—	—	—	1	0	0
Flaming Sword	Nov. 1943	20	8	9	—	—	0	—	—	—	—	0	1	0
Salmon Beauty	Nov. 1943	20	16	11	—	—	1	—	—	—	—	10	6	6
Lilac Wonder	Oct. 1944	180	27	7	—	—	—	0	—	—	20	—	—	—
Van Tienhoven	Oct. 1944	40	7	2	—	—	—	0	—	—	5	—	—	—
Prince of Wales	Oct. 1944	20	1	1	—	—	—	0	—	—	1	—	—	—
Copernicus	Oct. 1944	15	12	2	—	—	—	0	—	—	7	—	—	—
Foch	Oct. 1944	10	7	2	—	—	—	0	—	—	3	—	—	—
Vesuvius	Oct. 1944	8	1	1	—	—	—	0	—	—	1	—	—	—
Afterglow	Oct. 1944	5	5	2	—	—	—	0	—	—	4	—	—	—
Rose Precose	Oct. 1944	19	6	4	—	—	—	0	—	—	3	—	—	
America	Oct. 1944	3	3	1	—	—	—	1	—	—	1	—	—	
Ave Maria	Oct. 1944	40	11	—	—	—	—	—	0	—	—	—	—	—
Wolfgang v. Goethe	Oct. 1944	36	18	—	—	—	—	0	0	—	—	—	—	—

The figures in the table are numbers of corms rotting from *Botrytis* in storage.

extent dry rot (Hawker *et al.* 1944). Thus a single treatment applied in the autumn would probably reduce the incidence of four of the major diseases of the gladiolus. Owing to the efficacy of calomel against dry rot (Hawker *et al.* 1944) and scab (Miles, 1933), this fungicide was also tested but was relatively ineffective against *Botrytis* and harmful to the corms when applied in autumn.

(4) *Conditions of storage*

The stocks at Slough were in general stored in trays in an unheated shed and were covered with straw from December to March for protection against frost. These conditions were not ideal, and losses were much higher than among corms stored in open trays in a laboratory (heated by day) and covered with sacks only during periods of cold weather. Losses in the bulb shed were higher in large stocks piled several layers deep in the trays than in small experimental batches which were only one layer deep. No exact experiments were performed, but it is clear that losses would be reduced by storage in a single layer in a well-ventilated, dry, frost-proof store. Where storage in less ideal conditions is unavoidable, as at Slough, the fungicidal treatment described in the previous section becomes essential.

(5) *Fungicidal treatment of corms at planting time*

During work on hard rot and dry rot large numbers of corms were treated with various fungicides before planting (Hawker, 1944; Hawker *et al.* 1944). The results of a typical experiment are given in Table 8. Dusting with p.c.n. was the only treatment which also gave good control of *Botrytis*. The good effect continued throughout the growing season and the subsequent storage period. Treatment in March with mercurial fungicides (mercuric chloride, calomel and various proprietary compounds) gave little or no control of the disease, in contrast to the efficacy of an autumn application of mercuric chloride in preventing the development of the disease in stored corms. It is suggested that while mercuric chloride is more efficient than p.c.n. in killing spores, mycelium and possibly sclerotia of *Botrytis* on the corm, it is soon leached away when treated corms are planted and so is unable to protect them from attack by mycelium in the soil. The reduction in the numbers of old corms rotting in the soil in the lot treated with mercuric chloride is thus probably due to the killing of dormant mycelium or sclerotia which would otherwise have become active after planting. In contrast p.c.n., which is not readily soluble in water, remained as a protective layer over the old corms throughout the growing period. This volatile fungicide must also have had a protective influence over the young corms, since subsequent losses in storage were largely prevented. Calomel was ineffective when applied in the spring even though a deposit of the chemical was still visible on the old corms at lifting time.

TABLE 8. *Effect of fungicidal treatment of corms immediately before planting on losses from* Botrytis *during the growing season and the subsequent storage period*

Var. Rose Precose, 80 corms per treatment

Treatment	Losses of old corms in ground	New corms rotten when lifted	Corms rotting in storage
None	6	3	46
3 hr. 0·1 % $HgCl_2$	2	2	28
3 hr. 0·5 % Aretan	5	2	18
15 min. 0·5 % Aretan at 43° C.	7	2	23
Dusted p.c.n.	6	0	5
Dusted Ceresan	5	1	23

Table 9 summarizes all the experiments in which p.c.n. was used in this way. It has already been shown (Hawker, 1944) that no reduction in weight of new corms or delay in emergence or flowering follows such treatment.

Soil treatment with various fungicides (Formalin, mercuric chloride, Uspulun, Aretan, p.c.n. and Brassisan) did not significantly reduce the amount of disease.

Thus control of rotting due to *Botrytis* may be achieved by a combination of methods as follows: avoidance of wet or overshadowed ground or of ground where a diseased crop has been grown, rogueing of prematurely yellowing plants, early lifting followed by rapid drying of the corms (preferably at a temperature of about 20° C.), complete cleaning within a month of lifting, steeping in

TABLE 9. *Effect of dusting with p.c.n. before planting*

Variety	No. per treatment	Treatment	No. of old corms rotting in ground	No. of new corms rotten when lifted	No. of corms rotting in storage
Yvonne	20	None	0	0	13
	20	P.c.n.	0	0	0
Lilac Wonder	40	None	0	0	7
	40	P.c.n.	0	0	0
Rose Precose	80	None	6	3	46
	80	P.c.n.	6	0	5
Lilac Wonder	200	None	—	—	217
	200	P.c.n.	—	—	33
Rose Precose	80	None	16	11	41
	80	P.c.n.	11	5	16
Picardy	25	None	3	3	3
	25	P.c.n.	0	0	0
Afterglow	30	None	4	11	14
	30	P.c.n.	1	6	10
Lilac Wonder	40	None	1	0	14
	40	P.c.n.	0	0	9
Total	515	None	30	28	355
	515	P.c.n.	18	11	73

autumn for 20 min. in a 0·1 % solution of mercuric chloride, storage in a well-ventilated dry shed and dusting with p.c.n. before planting. The fungicidal treatments should also control hard rot, scab and to some extent dry rot.

Thanks are due to Mr T. W. Burrows who carried out preliminary experiments in 1938–9 and to Mr W. Buddin for material and advice.

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APPENDIX

Since this manuscript was completed, an account of an investigation of a disease of gladiolus corms caused by *Botrytis* sp. in Australia has been published (Wade, 1945), and publications describing a similar disease in Holland have been received (Timmermans, 1941, 1942). Timmermans describes a new species, *B. gladiolorum*, causing leaf spot, premature dying of plants and rotting of corms in storage, and suggests that storage rot can be reduced by early lifting and by quick drying at a temperature of 25–30° C. When warm storage conditions are not available he suggests that the stems should be torn off immediately after lifting. Wade reports that losses in storage were reduced by dipping corms for 2 min. in a 1 in 1000 solution of mercuric chloride plus Agral 2 or in solutions of Aretan or Semesan. Foliage spotting was controlled by spraying with Bordeaux mixture.

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Diseases of the gladiolus

IV. Note on the incidence and control of scab disease (*Bacterium marginatum* McCull.)

By Lilian E. Hawker, *Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London*

Stocks of imported gladioli which originally contained a proportion of scabbed corms showed very little carry-over of the disease during the eight growing seasons 1938–45. Carry-over was greatest on ground where gladioli had been planted for several successive years.

The incidence of the disease was greatest in the hot summer of 1940.

Treatment with mercurial fungicides just before planting time gave good control. Two non-mercurial fungicidal dusts were less effective.

Moore (1939) included scab in the four most important diseases of the gladiolus in this country; the others being storage rot associated with *Botrytis* spp., hard rot (*Septoria gladioli* Passer.) and dry rot (*Sclerotinia gladioli* Drayt.). He stated that the disease was prevalent among imported stocks but described experiments in which diseased corms gave rise to healthy plants although in wet soil plants were often severely attacked. He concluded that the severe leaf spotting and neck rot phases of the disease were less prevalent here than in America.

A number of stocks were obtained in 1938 and 1939 for use in experiments on diseases of the gladiolus at the Biological Field Station of the Imperial College of Science and Technology at Slough. Some of these included corms with scab lesions although dry rot and hard rot were more prevalent. Bray (1942) reported that with a condemned, imported stock of variety Rose Precose in which 15, 14 and 15 % of the corms showed lesions of scab, hard rot and dry rot respectively, carry-over of disease in 1938 was less with scab than with hard and dry rots.

Except in the hot dry summer of 1940 very little scab has occurred even in stocks originally infected, and in a number of experiments parent corms with scab lesions did not produce any diseased new corms. Thus in 1942 and 1943 there were only fifty-two and twenty-nine corms showing scab lesions out of 2000–3000 corms of various varieties. Most of these were from plots on which gladioli had been grown for several successive years. In a few experiments in a heated greenhouse the proportion of diseased corms producing diseased offspring was considerably higher.

Moore stated that the most satisfactory corm treatments for scab control were steeping in a 0·1 % solution of mercuric chloride for 8, 10 or 12 hr. as suggested by McCulloch (1924), or dipping for 5–10 min. in a suspension of calomel (1 oz. per gal.) as advocated by Miles (1933). It has been shown by Hawker (1944) that steeping for longer than 3 hr. in mercuric chloride is liable to injure the corms.

The incidence of scab was too low for any data on the control of this disease to be collected from the large number of fungicidal experiments primarily designed to control hard rot, dry rot and *Botrytis* storage rot (Hawker, 1944; Hawker *et al.* 1944; Hawker, 1946). Corms showing scab lesions were selected and treated with various fungicides before planting. The results of these experiments are given in Table 1. Experiments in 1942 and 1943 gave no results, since there was only a negligible amount of carry-over of the disease. No experiments were performed in 1945 since there were insufficient scabbed corms available.

The good control given by mercuric chloride is of importance, since this fungicide also gives good control of hard rot (Hawker, 1944) and fair control of dry rot (Hawker *et al.* 1944), while when applied in the autumn it controls losses in storage due to *Botrytis* (Hawker, 1946). The possibility of a single treatment to control these four diseases has obvious advantages. Calomel is superior to mercuric chloride in the control of dry rot but does not control hard rot or *Botrytis* storage rot (Hawker, 1944, 1946). Thus, except where the main trouble is dry rot, treatment of the corms with mercuric chloride would give the best all-round control of the main gladiolus diseases. Further experiments are in progress at Slough to determine the minimum strength of solution and duration of the treatment which will give effective control and also to determine conditions under which the treatment may be safely applied in

TABLE 1. *Effect of fungicidal treatments of corms with scab lesions before planting*

(Figures in table represent disease index, calculated by method described by Hawker (1944))

Variety	Season	No. of corms per treatment	Treatment							
			None	$HgCl_2$	Calomel	Aretan hot	Aretan cold	Ceresan	Folosan	Brassisan
Prince of Wales	1940	10	29·0	—	—	—	—	7·8	3·3	—
Yvonne	1940	10	50·0	—	—	—	—	4·0	6·7	—
	1940	20	3·5	—	—	—	—	0·5	—	—
Salmon Beauty	1940	40	3·8	0·5	—	1·3	1·3	—	—	—
Lilac Wonder	1940	25	4·4	—	—	—	—	2·4	2·4	0·8
Ave Maria	1940	20	19·0	—	—	—	—	9·4	13·0	16·0
	1941	60	2·0	0·0	0·2	—	—	—	—	—
Prince of Wales	1941	20	1·3	0·0	0·0	—	—	—	—	—
Lilac Wonder	1944	20	11·6	0·0	5·0	—	—	—	—	—

autumn. Autumn treatment is the only one effective in preventing storage rot (Hawker, 1946) and is as effective as spring treatment in controlling hard rot (Hawker, 1944), but no data are as yet available relating to the relative efficacy of autumn and spring treatment for the control of dry rot or scab. Moreover, experiments in the present series were always carried out with dehusked corms and it is desirable to test the effect of the mercuric chloride treatment on corms cleaned to the growers' standard, viz. with roots, cormels and old corms removed but with old leaf bases or husks, together with 2–3 in. of the old stem, intact.

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Further studies on the effect of disinfecting and bruising seed potatoes on the incidence of dry rot (*Fusarium caeruleum* (Lib.) Sacc.)

By T. Small, Ph.D., A.R.C.S.,
Agricultural Advisory Department, Manchester University

The effect on dry rot of the potato of disinfecting seed potatoes with a proprietary organo-mercury fungicide immediately before they were clamped on lifting, and on their removal from the clamps after 3 or 6 months' storage, has been tested in field trials during two seasons. The incidence of the disease in tubers stored in clamps has also been studied. The tubers used were of the susceptible varieties Ninetyfold and Doon Star, taken from crops grown in contaminated soil. In certain of the tests the natural contamination in the soil adhering to the tubers was reinforced by spraying each tuber with a spore suspension of the dry-rot fungus.

Seed tubers not deliberately bruised on lifting, and whether dipped or not at that time, were practically sound after 3 and 6 months' storage in clamps except in one instance. On removal from the clamps, those not dipped on clamping continued to remain sound when carefully handled and stored for a further period in seed boxes, but they suffered either moderate losses if slightly bruised, or serious losses if severely bruised, at the time they were removed from the clamps. Tubers dipped immediately before being clamped yielded similar results, except that they remained sound when slightly bruised on removal from the clamps.

Tubers not dipped, but deliberately bruised either on lifting in July immediately before clamping or on reclamping in October, developed severe dry rot whilst stored in the clamps. The incidence of the disease was not influenced by the location of the tubers within the clamp.

In tubers not dipped at lifting and clamping time in July, but lightly bruised and immediately dipped on their removal from the clamps in October, the losses from dry rot were slight. Severe bruising followed immediately by dipping on their removal from the clamp in October, however, resulted in considerable losses.

Tubers not dipped at lifting and clamping time in July but, after 3 or 6 months' clamp storage, slightly bruised and immediately dipped, then severely bruised 2 days later, developed appreciable losses; but these losses were far less than in tubers treated similarly except that the dipping was omitted.

Tubers dipped on lifting immediately before clamping in July, and slightly bruised and immediately redipped on removal from the clamps in October, remained almost sound; but tubers severely bruised just before the second dipping in October developed a loss of 12%.

All the foregoing results were obtained with tubers taken from healthy clamps. Ostensibly healthy tubers taken carefully from clamps containing much dry rot became severely affected with the disease when further stored in seed boxes.

Material and methods

In an earlier paper (Small, 1945) it was shown that when unbruised, naturally surface-contaminated seed potatoes were carefully lifted in July, stored in *boxes* until October, then dipped in a solution of Aretan,* and, lastly, severely bruised soon after dipping and returned to the boxes, they still remained practically free from dry rot until planting time in the following season in 5 out of 6 tests. The effect of such delayed disinfection (as opposed to that carried out immediately on lifting) has now been tested using for the purpose contaminated tubers that had been stored in *clamps* for 3 and/or 6 months after lifting in July. The results are given in the present paper which also provides data on the incidence of dry rot in seed potatoes stored in clamps, an aspect of the problem which has, as yet, received little attention. The trials were made at the University Field Station, Warburton, Cheshire, in the two seasons 1943–4 and 1944–5.

* A proprietary organo-mercury fungicide, already proved to be toxic to the dry-rot fungus.

Source of the tubers used

The very susceptible early variety Ninetyfold was used throughout, except in one test in which Doon Star, a late, susceptible one was employed. The Ninetyfold tubers were from a crop planted in late April, in fields the soils of which were known to be contaminated with the dry-rot fungus, and it was lifted with a potato plough in July, when the haulms were still green and the tubers immature. This crop was grown under the ordinary commercial conditions prevailing in the district, and received 10 tons of dung and 4 cwt. of artificials per acre. All the

tubers, except the chats, were gathered by hand into seed boxes in the field and were used at once for the trials recorded in this paper. The Doon Star crop was grown and handled in the same way, but it was not lifted until fully mature, viz. in late October, 3 weeks after the haulms had been removed to minimize blight (*Phytophthora infestans*) attack on the tubers.

Tuber treatments

(i) *Contamination.* Although the soil adhering to the tubers at lifting time was known to be contaminated with the dry-rot fungus, in certain of the trials the surface of each tuber was sprayed with a heavy spore suspension of the fungus immediately after lifting, to reinforce the natural contamination already present. Such tubers are referred to as 'heavily contaminated' as opposed to the 'natural contamination' present on the tubers at digging time. The suspensions were prepared from recently isolated pure cultures in tap-water to which a little raw tuber had been added. Spore viability was always verified by germination tests in hanging drops and by wound inoculations into susceptible healthy tubers.

(ii) *Bruising methods.* Apart from, and in addition to, slight unavoidable damage during lifting and handling, the tubers for some of the experiments were further injured by deliberate bruising. The 'standard bruise' was obtained by tipping each box of tubers into a 1 cwt. hamper, which was then shaken vigorously 30 times. When still greater bruising was desired, with the object of simulating the worst that would be likely to occur during transport by rail or by sea in sacks, the tubers (without any residual soil from the seed boxes) were transferred by hand to sacks, which were then vigorously shaken and trampled on. This treatment is referred to as 'severe bruising'. A third method of bruising, less severe than the others, was to grade the tubers with a hand riddle on their removal from the clamps.

The effects of these three bruising methods varied considerably from one trial to another, and for this reason the extent of the injury caused in the various experiments will be noted in the appropriate context. The terms bruised, not bruised or unbruised refer exclusively to the above maltreatments or their absence, and not to slight accidental damage inseparable from the methods employed in raising and handling the tubers.

(iii) *Disinfection.* The dipping process has already been fully described in a previous paper. (Small, 1945). Briefly, it consisted of immersing the Ninety-fold tubers in their boxes for 1 min., those of Doon Star for 3 min., in a 0·5% solution of Aretan. Heavily contaminated tubers were not dipped until at least 3 hr. after they had been sprayed with the spore suspension. It was found that tubers with the soil that had dried on them after storage for some weeks in boxes and clamps were far more difficult to wet thoroughly than freshly dug still damp or deliberately bruised ones, for small dry areas often persisted on them.

(iv) *Clamping.* All the tubers were clamped on the day they were dug, except the dipped ones; these were allowed to dry in their boxes overnight and were clamped next day. Unbruised tubers were carefully tipped from the boxes into the clamps. Clamps made in July were at first covered only with straw and hedge clippings but soil was added in October. All the lots of tubers included in one and the same trial were stored in the same clamp, but they were separated by straw partitions.

(v) *Precautions against recontamination.* The precautions previously found adequate and noted in an earlier paper (Small, 1945) were taken. In addition, the hand riddles were soaked in Aretan after each batch of tubers had been riddled, whilst at clamping time, dipped tubers were placed on clean straw at the base of the clamp. When the clamps were opened for examination, the diseased tubers were removed and counted by one person, and the healthy ones by another who put them by hand into new seed boxes, or into old boxes or hampers previously soaked in Aretan.

(vi) *Presentation of results.* The figures summarized in the Tables represent the total percentage losses of tubers from dry rot (by number) for each series, and since the trials were on a large scale, these percentages may be regarded as based on adequate numbers of tubers. Losses below 1% are entered as nil.

Large-scale field trials such as those now under consideration are of course subject to somewhat extensive experimental errors, nevertheless, it is believed that they do not invalidate the main conclusions drawn from the results. In certain of the trials, however, the data presented are too meagre to permit of definite conclusions being drawn. The writer was anxious to repeat and extend some of his experiments but owing to unforeseen circumstances this has now become impossible. He considers, however, that the results should be recorded for the benefit of future workers on this important disease.

Results

(1) *Comparative incidence of dry rot in tubers stored in clamps and in seed boxes*

In two trials, in 1943–4 and 1944–5 respectively, naturally and heavily contaminated freshly dug tubers were either standard bruised on lifting in July or left unbruised, and stored at once in clamps and boxes. The total numbers of tubers in the clamps and the boxes, respectively, were 4405 and 1003 in 1943–4

and 4427 and 1192 in 1944–5. Both lots were examined on 1 September 1943, and 28 September 1944, and the tubers affected with dry rot were removed. The healthy ones remaining in the boxes were left undisturbed, and were re-examined in November; a further examination made a month later showed no increase in the amount of dry rot. The healthy tubers in the clamps opened in September were carefully reclamped and allowed to remain undisturbed until January of the following year. The percentages of tubers affected with dry rot found at the various times of examination are given in Table 1.

The large amount of dry rot present in the bruised tubers on 1 September 1943, 6 weeks after lifting, shows how quickly the disease may develop in immature tubers, and the losses were higher in the heavily than in the naturally contaminated tubers.

The effect of reclamping on the incidence of dry rot will be discussed in the next section, but it may be pointed out here that, in each year, the opening of the clamp and the reclamping of the tubers in September did not cause increased disease in the unbruised, naturally contaminated tubers in Series 1. The increase in the other series was considerable in

TABLE 1. *Percentages of dry rot in Ninetyfold tubers stored in clamps and seed boxes*

		1943–4					
		Clamp			Seed box		
Series	Treatment at lifting time in July	1. ix. 43	28. i. 44	Total	1. ix. 43	2. xi. 43	Total
1	Natural contamination; not bruised	0	1	**1**	0	0	**0**
2	Heavily contaminated; not bruised	1	10	**11**	0	2	**2**
3	Natural contamination; standard bruised	17	5	**22**	9	3	**12**
4	Heavily contaminated; standard bruised	37	16	**53**	38	5	**43**

		1944–5					
		Clamp			Seed box		
Series	Treatment at lifting time in July	28. ix. 44	30. i. 45	Total	28. ix. 44	2. xi. 44	Total
1	Natural contamination; not bruised	2	0	**2**	0	0	**0**
2	Heavily contaminated; not bruised	3	1	**4**	1	0	**1**
3	Natural contamination; standard bruised	4	1	**5**	9	0	**9**
4	Heavily contaminated; standard bruised	10	2	**12**	37	0	**37**

From the figures given in Table 1 it can be seen that when naturally surface-contaminated immature tubers, even of a susceptible variety like Ninetyfold, were raised and handled with reasonable care, and stored for 5 months with a minimum amount of disturbance in either clamps or seed boxes, the amount of dry rot that developed was practically negligible (Series 1). Under similar conditions such tubers with reinforced surface contamination (Series 2) developed considerably more disease in the clamp in 1943–4, but only slightly more in the clamp in 1944–5 and in the seed boxes in both years. In both series the amount of disease was greater in the clamps than in the seed boxes but, with the exception of the 1943–4 clamp, the total amount of disease was relatively slight.

Bruising at lifting time, immediately before boxing or clamping (Series 3 and 4), increased the losses appreciably both in the box-stored and clamp-stored tubers except in one instance. The data, although inconclusive as to the relative incidence of dry rot in boxes as compared with clamps, show that heavy losses may occur under both methods of storage when the seed tubers are roughly handled. 1943–4 but only slight in 1944–5. The former increase was probably due to the early examination, which had not allowed sufficient time for all the disease to become obvious by the time they were first examined early in September.

Location of diseased tubers in the clamp. When examining Series 3 and 4 in September 1943, the diseased tubers were found to be distributed fairly uniformly throughout the clamps. This was of interest because of a widespread belief among growers that dry rot occurs mainly in the outer part of a clamp. Since, however, the clamps here described were not typical, in that they were covered only with straw and hedge clippings from July to October, the following trial was made. 843 healthy Ninetyfold tubers were removed by hand from a healthy clamp on 3 November 1943, and sprayed at once with a spore suspension of the dry-rot fungus. Half of them were then standard bruised, the other half being left unbruised. Each half was then placed in three new seed boxes. The bruising caused extensive skin abrasions. After encasing each box in 1 in. mesh wire netting, two of them containing bruised and one containing unbruised tubers were placed in the interior, near the base, of a large commercial clamp of Majestic potatoes which was then being prepared, whilst the three remaining boxes were built into the outer

layer of the same clamp, which was completed and covered with straw and soil on the day mentioned. To determine whether the tubers in the buried boxes were sound at the start of the experiment, an additional 250 tubers were taken on the same day from the same source as the experimental tubers and were stored, unbruised, in a seed box in the laboratory; only three of them developed dry rot up to May 1944.

The boxes were removed from the Majestic clamp on 25 January 1944, and the results of the examination made on that date are given in Table 2.

TABLE 2. *Dry rot in heavily contaminated Ninetyfold tubers stored in the interior or in the outer part of a potato clamp*

Treatment and storage of tubers, 3. xi. 43	Dry rot (%) 25. i. 44
Unbruised; in interior	8
Unbruised; in outer part	7
Standard bruised; in interior	87
Standard bruised; in outer part	96

The results show that the conditions within the clamp during the 12 weeks were very favourable for the disease, and that the location of the tubers had no differential effect on its incidence. The very serious effect of bruising is once more clearly evident.

tubers were left unbruised whilst others were severely bruised, then all were reclamped and stored till the following January when they were re-examined. Bruising caused cracks in a few tubers but skin abrasions were not extensive. Other clamps were opened and examined for the first time in January, after being closed and undisturbed for 6 months, a period seldom exceeded in practice. The various treatments and the percentages of dry rot recorded at each examination are summarized in Table 3.

In each trial the loss in October, after 3 months' storage, in the dipped and undipped tubers was negligible except that of 6% in Series 5 of 1943–4. Those in January, after 6 months' undisturbed storage, were 4% and nil in the dipped tubers, and 13% and nil in the undipped. The 13% loss cannot perhaps be regarded as excessive since each tuber had been heavily contaminated.

Reclamping of undipped healthy tubers in October did not cause dry rot to develop except where the tubers were deliberately bruised at that time. This, considered together with the results given in Table 1, strongly suggests that bruising at reclamping time was the main cause of the very substantial increase of dry rot in the clamps.

TABLE 3. *Effect of dipping and of reclamping on the incidence of dry rot in clamp-stored Ninetyfold tubers*

Series	Treatment before clamping in July	Examination and further treatment	Dry rot (%) 1943–4 Oct.	1943–4 Jan.	1944–5 Oct.	1944–5 Jan.
1	Dipped	Examined in October	1	—	0	—
2	Dipped	Examined in January	—	4	—	0
3	Not dipped	Examined in January	—	13	—	0
4	Not dipped	Examined in October, reclamped but not bruised; re-examined in January	—	—	0	0
5	Not dipped	Examined in October, severely bruised and reclamped; re-examined in January	6	37	0	70

(2) *Effect of dipping at lifting time and of either continuous or interrupted clamp storage on the incidence of dry rot*

In these trials, all the tubers were heavily contaminated on the day of lifting in July, and either clamped, undipped, on that day or dipped at once and clamped when dry on the next day. They were not deliberately bruised. The minimum number of tubers included in any one series at any time was 1135, but in some series as many as 4000 tubers were used. Certain of the clamps were first opened and examined in October, 3 months from lifting time, and all the tubers affected with dry rot were discarded. After this some of the remaining healthy

(3) *Effect of dipping and bruising seed tubers after 3 or 6 months' storage in clamps on the incidence of dry rot*

For these trials, tubers which appeared to be quite healthy were selected from the clamps referred to in Tables 1 and 3 at the time these were opened, either in October, after 3 months', or in January, after 6 months' storage. The source and the treatment of the tubers up to the time they were used for these trials are outlined in the appropriate section. In all the tests riddling by hand caused only slight damage; severe bruising crushed a few tubers and caused fine cracks to appear in a few others, but did not result in extensive skin abrasions. In general, the damage was

far less than that produced by similar bruising treatment on freshly dug immature tubers in July.

A. *Effect on dry rot of dipping and bruising seed tubers after 3 months' storage in clamps*

(*a*) *Trials with Ninetyfold.* The tubers used were from the three Series 1, 4 and 5 of Table 3, taken from the clamps in October. All had been heavily contaminated, but not bruised, at lifting time in July, and then either clamped at once (undipped) or dipped and then clamped the next day. The losses in October, after 3 months' clamp storage, had been practically negligible.

From the 3 months old clamps the tubers were put by hand into new seed boxes and immediately subjected to the various treatments shown in Table 4. Thereafter they were stored in new seed boxes in a clean shed, the floor of which was frequently sprayed with Aretan. The average number of tubers used in each test in each Series in 1943–4 was 343, and in 1944–5 was 489.

The dry soil adhering to the unbruised and the riddled tubers was difficult to wet thoroughly when they were dipped and small dry areas persisted on many of them. On the contrary, tubers severely bruised immediately before dipping became, as a result, completely wetted with the fungicide.

The precautions to avoid recontamination referred to on p. 212 were taken and, in addition, as a consequence of the results obtained in 1943–4, each box of seed tubers in the 1944–5 trial was kept covered with newspaper. The percentage losses due to dry rot in the storage period October to March 1943–4 or October to April 1944–5 are given in Table 4.

Series 1 and 2 in Table 4 show that unbruised, heavily contaminated tubers, dipped or not dipped at digging and clamping time in July, carefully removed from the clamps in October and afterwards stored in seed boxes till near planting time in March or April, remained practically sound whether dipped in October or not. The results of Series 1 and 2 also show that the tubers used in the five Series 3 to 7, must have been sound on removal from the clamps in October and before they were subjected to further treatment at that time.

Comparison of Series 1 with Series 3 and 6 shows the effect of riddling and severe bruising in October on tubers stored in clamps since July. The slight damage caused by hand riddling (Series 3) resulted in losses of 19 and 15% in tubers undipped at clamping time, but in almost no loss (3 and 0%) in those dipped at that time. Severe bruising in October (Series 6) caused losses of 69 and 84% in tubers undipped at clamping time, and of 31 and 30% in those dipped at that time.

The effect of dipping immediately after riddling in October is shown in Series 3 and 4. In tubers not dipped on clamping in July, but riddled and dipped on removal from the clamp in October, the losses were reduced from 19 to 11% in 1943–4 and from 15 to 3% in 1944–5. In tubers not dipped on clamping in July and subjected to severe bruising two days after riddling and dipping in October (Series 5), the losses were 37 and 13%. In tubers dipped on clamping in July and then riddled and redipped in October, the losses were negligible in each year.

The effect of dipping immediately after severe bruising in October is shown by comparing Series 6 and 7. In tubers undipped on clamping in July, and severely bruised and dipped at once in October, dry rot was reduced from 69 to 51% in 1943–4, and from 84 to 37% in 1944–5, whereas in tubers stored and treated in a similar manner except that they were dipped on clamping in July, dry rot was reduced from 30 to 12%.

Series 5, when compared with Series 6, shows that dipping in October, 2 days before the tubers were severely bruised, reduced the losses from 69 to 37% in 1943–4, and from 84 to 13% in 1944–5.

TABLE 4. *Effect of dipping and bruising Ninetyfold tubers at the end of 3 months' clamp storage (July to October) on the percentage of dry rot appearing during further 6 months' storage in boxes*

Series	Treatment of tubers on removal from clamps in October	Treatment of tubers before clamping in July: Not dipped — Date tubers examined: 8. iii. 44	Dipped — 8. iii. 44	Not dipped — 12. iv. 45	Dipped — 12. iv. 45
1	Not riddled; not dipped	4	1	0	0
2	Not riddled; dipped at once	2	—	1	—
3	Riddled; not dipped	19	3	15	0
4	Riddled; then dipped at once	11	0	3	0
5	Riddled; then dipped at once and severely bruised after 2 days	37	—	13	—
6	Severely bruised; not dipped	69	31	84	30
7	Severely bruised; then dipped at once	51	—	37	12

(*b*) *Trial with Doon Star.* The tubers used were grown in contaminated soil at Warburton under the conditions outlined earlier except that at planting time the seed tubers planted in one series were healthy whilst those in the other were already partly decayed with dry rot. The crop was carefully lifted with a potato plough on 30 October 1944, three weeks after the haulms had been removed and when the skins of the tubers were firm. All except the very small tubers were gathered by hand into 1 cwt. hampers from which they were gently tipped into the clamp on the day they were lifted. The clamp was covered at once with straw and soil, a straw partition inside separating the two series. The tubers were merely naturally contaminated and they were neither bruised nor dipped before being clamped. When the clamp was opened for the first time, in January 1945, after 3 months' storage, the loss from dry rot was less than 1 %. At this time three lots of 800 tubers each were selected from each series, treated as shown in Table 5, and afterwards stored in boxes till May. The methods of treatment and the effects of the riddling and bruising to which some of them were then subjected were similar to those already described for the corresponding series given in Table 4, except that the tubers were immersed in the fungicide for 3 min. instead of 1 min. The total percentage losses at each examination are given in Table 5.

The result in Series 1 agrees with that of the corresponding series (Series 2) in Table 4, and suggests that little if any infection occurred in the unbruised, undipped naturally contaminated tubers during clamp storage from October to January and box storage from January to May. In Series 2, riddling followed immediately by dipping, and then by severe bruising two days later, caused losses of 4 and 13 %, whereas in Series 3, severely bruised but not dipped, the losses were 67 and 97 %. In Series 2 and 3 more dry rot occurred in the tubers grown from seed tubers already affected with the disease possibly because the soil adhering to them was more heavily contaminated. The appearance of more dry rot at each successive examination, a result frequently obtained in these studies, is well illustrated in Table 5 and will be discussed later.

TABLE 5. *Effect of dipping and bruising Doon Star tubers at the end of* 3 *months' clamp storage (October to January), on the percentage of dry rot appearing during further* 5 *months' storage in boxes*

Series	Treatment of tubers on removal from clamp in January	Tubers grown from healthy seed tubers, dug and clamped, but not dipped, in October. Removed from clamp in January and treated as shown in column 1 — Dates of examination of tubers			Tubers grown from diseased seed tubers, dug and clamped, but not dipped, in October. Removed from clamp in January and treated as shown in column 1 — Dates of examination of tubers		
		12. iv. 45	1. v. 45	22. v. 45	12. iv. 45	1. v. 45	22. v. 45
1	Not riddled; dipped	1	1	2	1	2	2
2	Riddled, dipped at once; severely bruised 2 days later	1	3	4	7	11	13
3	Severely bruised; not dipped	41	61	67	35	63	97

B. *Effect on dry rot of dipping and bruising seed tubers after six months' storage in clamps*

In view of the results obtained in 1943–4 three experiments were carried out in 1944–5 with Ninety-fold tubers that had been clamp-stored for 6 months, from July to January, a period unlikely to be exceeded in practice. All the tubers used had been heavily contaminated at lifting time, except those in one section of exp. 3, and were taken from healthy clamps except those for exp. 3. The treatment of the tubers up to the time they were used in these three experiments was as follows:

Exp. 1. The tubers were taken from the clamp of 1944–5, Series 4, Table 3. On lifting in July 1944, they had been carefully clamped at once, but not dipped. They were examined in October and immediately reclamped carefully and further stored until January 1945. Then 872 sound tubers were selected for this experiment.

Exp. 2. The tubers were taken from the clamp of 1944–5, Series 2 and 3, Table 3. Those of Series 2 had been dipped a few hours after lifting in July and carefully clamped the next day, while those of Series 3 had been carefully clamped, undipped, on the day they were lifted. The clamps were first opened in January 1945, and 2318 healthy tubers were selected for this experiment.

Exp. 3. The tubers were taken from the 1944–5 clamp, Series 1–4, Table 1. Those of Series 2 and 4, but not those of 1 and 3, had been heavily contaminated on lifting in July, immediately after which Series 3 and 4, but not Series 1 and 2, had been standard bruised. No dipping was carried out. All the Series had been clamped on the day they were lifted. The clamp was opened in September 1944, and the healthy tubers were at once carefully reclamped and further stored until January 1945, when

3568 healthy tubers were chosen for this experiment. Reference to Table 1 will show that the losses in Series 1–4 in the clamps were 2, 4, 5 and 12% respectively.

On removal from the clamps in January all the tubers for the three experiments were placed by hand in new seed boxes and immediately treated as shown in Table 6. In Series 2, but not in Series 4, small dry areas were visible on many of the tubers after they had been dipped, for the reason already given. The percentages of dry rot on 2 May, after 13 weeks storage in seed boxes, are given in Table 6. In that Table, the only tubers that had been dipped at clamping time in July are those shown in exp. 2, column B; in addition, the columns 1 to 4 shown there under exp. 3 correspond with the Series 1–4 in the 1944–5 clamp of Table 1, from which series the tubers were taken.

(4) *Incidence of dry rot in ostensibly healthy tubers after removal from clamps containing much dry rot and during further storage for 20 weeks in boxes*

Almost all the contaminated tubers used in the trials summarized in Tables 4, 5 and 6, were taken from practically healthy clamps, and, as already shown, they remained sound during further storage in boxes for various periods after their removal from the clamps provided they were carefully handled. The question arose, and has been investigated in the three experiments now to be described, as to whether tubers, apparently healthy at the time they were removed from clamps containing much dry rot, would continue to remain sound for a further period when stored in seed boxes. The tubers used were selected from three different clamps in which they had been stored for 6 months (July to January)

TABLE 6. *Effect of dipping and bruising Ninetyfold seed tubers at the end of 6 months' clamp storage (July to January) on the percentage of dry rot appearing after 13 weeks' further storage in seed boxes*

		Results on 2. v. 45						
		Exp. 1	Exp. 2		Exp. 3			
Series	Treatment of tubers on removal from clamps in January 1945		A	B	1	2	3	4
1	Not riddled; not dipped	1	2	1	—	—	—	—
2	Not riddled; dipped at once	2	1	0	1	4	2	4
3	Severely bruised; not dipped	92	96	98	93	94	92	92
4	Riddled, dipped at once, severely bruised after 2 days	12	29	4	10	24	42	35

Series 1 and 2 show that the tubers were practically healthy when taken from the clamps in January, after 6 months' storage, and that they remained so for a further storage period of 13 weeks in seed boxes whether dipped in January or not. The results agree with those already given for Series 1 and 2 in Table 4, and for Series 1 of Table 5.

The disastrous effect of the severe bruising on removal of the tubers from the clamps in January, is shown in Series 3. In tubers that had been dipped on clamping in July (exp. 2, column B) as well as in those not dipped at that time, severe bruising on removal from the clamp in January 1945 caused losses exceeding 90% without exception.

The effect of dipping in January 1945 immediately after the tubers were riddled and 2 days before they were severely bruised, was to reduce the losses considerably, although substantial losses were still incurred particularly in tubers not originally dipped at clamping time in July. In general, these results agree with those of the corresponding series in Tables 4 and 5.

and in which the losses from dry rot up to then had been 37, 53 and 70%, respectively. All of them had been severely bruised either when clamped in July or when reclamped in October. Further information on the sources of the tubers is given in Table 7, to enable their treatment to be traced up to the time they were used in these last experiments. On removal from the clamps in January, they were placed without dipping or bruising in new seed boxes, in which they were stored for 15 or 20 weeks. Altogether 1328 tubers were used.

The total percentage losses found at each successive examination are given in Table 7. Severe losses of 38, 61 and 41% were sustained, a result in sharp contrast with that obtained with tubers from healthy clamps. Clearly, the tubers must either have been infected, but not visibly, at the time they were removed from the clamps or have become infected subsequently. That fresh infections may have occurred from time to time whilst the tubers were stored in the boxes is suggested by the fact that more dry rot became evident at each successive examination,

TABLE 7. *Incidence of dry rot in apparently healthy Ninetyfold tubers subsequent to their removal from clamps containing much dry rot, and storage in boxes for a further period of 20 weeks*

Exp.	Source of tubers	Percentages of dry rot after the tubers had been stored in boxes for 9 weeks	12 weeks	15 weeks	20 weeks
1	Taken 20. i. 44 from Series 5 of 1943–4 clamp, Table 3	15	—	29	38
2	Taken 28. i. 44 from Series 4 of 1943–4 clamp, Table 1	—	10	30	61
3	Taken 30. i. 45 from Series 5 of 1944–5 clamp, Table 3	24	29	41	—

and that at the final one, made 15 to 20 weeks after the start of the experiments, many dry-rot lesions were no more than ½ in. across. In this connexion, however, it should be pointed out that in laboratory tests, under controlled conditions, and also in field trials, dry rot has frequently been observed during the course of these studies to develop at very different rates in different tubers of the same series.

The results are of practical interest because in one respect they are reminiscent of what happens frequently in fresh Scotch or Irish seed after it has been received and placed in boxes by the English grower. Here as in the above experiments dry rot continues to appear over a period of maybe several months up to planting time. These observations are markedly different from those reported in earlier investigations (Small, 1945) in which, in Ninetyfold tubers, dug immature in July and stored throughout in seed boxes almost all the disease developed quickly and had run its course by mid-October, provided that the tubers were not further handled after that date.

(5) *Practical considerations*

Effect of dipping tubers on removal from clamps. In the present investigations it has been shown that naturally contaminated, unbruised tubers, even if undipped, remained practically healthy when harvested with reasonable care and placed in clamps at lifting time in July and stored there for 3 or 6 months; also that they continued to remain so during further storage in seed boxes after removal from the clamps. These results suggested that theoretically, at least, the dipping of such surface-contaminated tubers at the time they were removed from the clamps might be expected to prevent dry rot even if the tubers were subsequently subject to mechanical damage. Dipping tests were therefore so designed as to simulate the conditions likely to obtain in commercial practice, in that the tubers, on removal from the clamps, were first graded (a hand riddle being used because a machine grader was not available), then dipped at once, and lastly, when dry, were placed in sacks and bruised as severely as would be likely to occur during transport by rail or sea in sacks. Although the dipping reduced the losses considerably in Ninetyfold in every trial, losses of from 10 to 42% occurred even in dipped produce. The results cannot be regarded as satisfactory for practical purposes but it should be noted that similarly stored and treated, but not dipped, tubers, suffered losses of from 69 to 96% thus showing that the tubers were subjected to a very severe test. Nevertheless, it is difficult to explain the losses after dipping, because in an earlier paper (Small, 1945) it was shown that dipping killed most, if not all, of the fungus in the soil adhering to freshly dug tubers, and that the disinfection of freshly dug tubers or of tubers which had been stored for 3 months (July to October) in seed boxes, followed soon afterwards by severe bruising, resulted in negligible losses. Further work may show that tubers removed from clamps require immersion in the fungicide for longer than 1 min., or that very vigorous agitation of them during immersion may be necessary to disinfect them thoroughly and thus give effective control of dry rot.

It should also be noted here that the results of the dipping tests described in this paper were obtained in trials with tubers taken from practically healthy clamps, and they were not infected at the time they were removed from the clamps. Further work may show that the dipping of ostensibly healthy tubers taken from clamps containing much dry rot, may yield still more disappointing results, because, in one of the trials described, such tubers developed a very serious amount of dry rot after their careful removal from the clamps and during further storage in boxes.

Effect of dipping at clamping time. Tubers dipped at lifting time immediately before they were clamped, but severely bruised 3–6 months later on removal from the clamps, developed serious dry rot on further storage in boxes. These results agree with those given in an earlier paper (Small, 1945) obtained by bruising dipped tubers after they had been stored in boxes in lofts for 3 months. For the latter results, several explanations might have been given, but the one considered most likely was that recontamination had possibly occurred from the lofts during storage. This explanation, however, cannot be applied to the results of the clamp trials reported here.

In contrast with the above, tubers dipped on clamping and lightly damaged 3 months later by

riddling, remained practically sound, whilst those treated in a similar manner but not dipped on clamping, developed losses of 15 and 19%.

The data presented in this paper are insufficient to decide the value of dipping at clamping time as a preventive of dry-rot development at a later period. It has been shown, however, that dipping at that time did not afford protection against severe bruising 3 or 6 months later.

Incidence of dry rot in clamps. In these investigations the losses from dry rot in naturally or heavily contaminated, unbruised, undipped tubers, after 3 or 6 months' undisturbed storage in clamps, were negligible except in a few instances. Similar results were obtained when the tubers were stored in clamps for 3 months, and then carefully reclamped and stored for a further 3 months. It was proved that these tubers were, in fact, healthy at the time they were removed from the clamps, so that little or no infection occurred during the period of clamp storage. On the other hand, similar tubers bruised on clamping or on reclamping developed serious dry rot in the clamps. Thus, the conclusion, based on tests with continuously box-stored tubers, reached earlier (Small, 1945), that little or no dry-rot infection occurs unless and until the tubers are bruised, would appear to hold good also for clamp-stored tubers. It is also clear that, contrary to the supposition of Foister (1940), the conditions within a clamp do not preclude the development of dry rot there and that the mere opening of a clamp or reclamping of the tubers does not increase the amount of disease except where the tubers are bruised.

The writer is indebted to Dr G. H. Pethybridge for help in preparing this paper.

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Dry rot of potato (*Fusarium caeruleum* (Lib.) Sacc.) Effect of planting infected and contaminated sets on plant establishment

BY T. SMALL, PH.D., A.R.C.S.,
Agricultural Advisory Department, Manchester University

The effect on plant establishment of planting infected and contaminated sets of susceptible varieties has been studied in field trials from 1942 to 1945.

Healthy whole sets, and whole sets inoculated internally 3 days before planting, yielded good stands, whilst whole sets with obvious dry-rot lesions present at planting time produced gappy crops.

Healthy cut sets gave satisfactory stands except in one test with Doon Star, whilst sets cut with a contaminated knife at planting time yielded only a few misses in 1943 but many in the unfavourable 1944 season and in 1945.

INTRODUCTION

In recent years dry rot of the potato has often been held responsible for gappy crops, and many farmers believe that such crops may occur even when healthy sets have been planted. The problem has been studied in field trials from 1942 to 1945 on light soil (*p*H 5·16–5·85) at Warburton, Cheshire, an area which is not early and which is subject to late spring frosts. The seasonal conditions were average in 1942 and 1945 but with heavy spring rains in 1945. In 1943 and 1944 they were favourable for rapid growth, but in the latter year severe May frosts killed all the shoots above ground and in this respect the variety Ninetyfold suffered far more severely than Doon Star.

VARIETIES, MANURING, PLANTING

The early variety Ninetyfold, the early maincrop variety Majestic, and the maincrop variety Doon Star were used, all three being susceptible to dry rot, especially the first and the last. Each series of each variety included four plots each of twenty-five sets

planted in the third week of April, at which time the shoots did not exceed 1½ in. in the Ninetyfold or ½ in. in the other varieties. Since the stand probably depends largely on the rates of plant establishment and of disease development in the sets after planting, no attempt was made to force crop growth. To this end fresh Scotch seed having short shoots was used, planting was done as early as was considered safe for the area, and only moderate dressings of stable manure (10 tons/acre) and of a standard potato fertilizer (4 cwt./acre) were applied. The sets were planted 14 in. apart in drills on top of the manure and fertilizer, and were covered at once. Subsequent cultivations followed the usual practice, but the final ridging was delayed until after the final plant counts had been made 8–9 weeks after planting.

Treatment of sets before planting

(i) *Whole sets.* Healthy sets were planted as controls. In the other two series the sets were inoculated internally 3 and 28 days respectively before planting, by removing a cone-shaped piece of tissue, placing the inoculum (taken from pure cultures of the dry-rot fungus) in contact with the flesh, and afterwards replacing the cone. Since in practice the result is probably affected by the position of the dry-rot lesion relative to the shoots, and since natural infections may occur at any place on the tuber, the inoculations were made midway between the rose and heel ends of the sets. In sets inoculated 28 days before planting, the external lesions were 1–1½ in. across at planting time. Two-oz. sets were used in each trial except for the 3-oz Majestic ones in 1942.

(ii) *Cut sets.* In an earlier paper (Small, 1944) it was shown that a seven-fold increase in dry rot occurred when seed tubers were cut with a contaminated knife. Where the disease is spread on cutting the sets several weeks before planting, it is likely to be so obvious later that the diseased sets can be discarded before planting begins. But when the sets are cut on planting, a common practice in this district, the question arises and has been studied here, whether the dry rot carried by the knife can cause misses in the crop. In these tests healthy 4-oz. seed tubers were cut into halves at planting time, using a sterilized knife (controls) or one which had been contaminated by cutting a dry-rot tuber before each healthy seed tuber was cut.

Examination of sets after planting

To obtain information on the progress of the dry rot in the sets after planting, twenty-five sets of each series of each variety were planted in 1943–5 alongside the main plots, and were dug and examined 5 weeks later (8 weeks later in 1944 because of the May frosts). The results were as follows. Of the whole sets, almost all the controls were sound. None of those inoculated 3 days before planting was healthy, but usually no more than one-half of each set was diseased except in 1944 when all the Ninetyfold were completely rotted. In those inoculated 28 days before planting, little healthy tissue remained each year and especially in 1945.

Of the control cut sets several were sound, but many had begun to decay, and in the Doon Star in 1945, eighteen of them were completely decayed. Almost all the sets cut with a contaminated knife were completely rotted in 1944 and 1945, but the rot was less advanced in 1943.

The above observations showed that all the treated sets decayed more rapidly than the controls, and this was especially noticeable in the wet spring of 1945. The decay of the control cut sets resembled that of dry rot but no isolations were made; it may have been contracted from the field soil or from the contamination probably present on the outside of the tuber at cutting time. It seems reasonable to conclude from these examinations that all the internally inoculated whole sets and all the deliberately contaminated cut sets planted in the main plots developed dry rot after planting. The results from these plots will now be given.

Results and practical considerations

The numbers of misses and small plants occurring at the final counts, made 8–9 weeks after planting, when growth was well advanced, are given in Tables 1 and 2. In these tables the first figure in each column of each series denotes the number of misses and the second the number of small plants. A miss was recorded when little or no growth was visible. Usually, although there was considerable growth on the small plants it was not difficult to distinguish them from normal plants and the numbers of borderline cases were considered too few to affect the general results. In considering the results it should be remembered that they may not apply to crops grown on heavy soils, and that each set, except the controls, was infected or contaminated before it was planted, whereas in practice most of the sets used would, presumably, be healthy when planted.

(1) *Results with whole sets.* Table 1 shows that healthy sets, and sets inoculated 3 days before planting, yielded good stands each year. Those inoculated 28 days before planting produced gappy crops each year except Ninetyfold in 1942, and, except in 1944, the number of misses was lower in Ninetyfold than in the later varieties, possibly because the latter established themselves more slowly. The exceptional result in 1944 was probably due to the above ground shoots of Ninetyfold being far

TABLE 1. *Effect of planting internally inoculated whole sets;* 100 *sets of each variety planted each year*

	No. of misses and small plants								
	1942		1943			1944		1945	
	Ninety-fold	Majestic*	Ninety-fold	Majestic	Doon Star	Ninety-fold	Doon Star	Ninety-fold	Doon Star
Healthy (control)	1+7	0+2	0+0	1+5	0+4	2+0	5+4	0+1	5+6
Inoc. 3 days before planted	1+10	2+2	4+7	2+6	2+0	5+1	2+6	0+4	5+7
Inoc. 28 days before planted	0+6	48+18	8+2	48+20	12+8	43+2	13+2	18+9	42+20

* 3 oz. size sets.

TABLE 2. *Effect of planting contaminated cut sets;* 100 *sets of each variety planted each year*

	No. of misses and small plants					
	1943		1944		1945	
Treatment of seed tuber	Ninetyfold	Majestic	Ninetyfold	Doon Star	Ninetyfold	Doon Star
Cut with sterilized knife (control)	0+3	0+3	0+3	4+7	3+4	19+6
Cut with contaminated knife	3+5	4+10	16+20	20+22	11+11	39+11

more advanced than those of Doon Star when the May frosts killed them.

The results suggest that satisfactory stands are likely to be obtained when healthy whole sets are planted in contaminated soil, even if, as is shown by the series inoculated 3 days before planting, the sets become infected soon after planting. The use of many obviously diseased sets may result in poor stands, but this is likely to happen only where dry rot has been prevalent in the seed tubers and where the final sorting in the boxes has been faulty or carried out so early that much disease has developed subsequently and before planting took place. In an earlier paper (Small, 1944) the writer showed that in Ninetyfold seed tubers dug in July in Cheshire and box stored continuously, dry rot had run its course by late October, and he suggested that sorting may be safely carried out any time after that month. Where, however, the disease continues to appear in the boxes up to planting time, as it frequently does in fresh Scotch or Irish seed stored in boxes in England, it may be preferable to delay sorting until as near planting time as possible.

(2) *Results with cut sets.* In each year the healthy control sets planted in contaminated soil yielded good stands, except in Doon Star in 1945 when the sets of this variety, possibly due to the wet spring, decayed rapidly after planting. Sets cut with a contaminated knife suffered slightly in 1943 and severely in 1944 and 1945, but it should be noted that contrary to practice, the knife was used to cut a diseased tuber before *each* sound set was cut. It seems doubtful if dry rot, induced by cutting on planting, would cause gappy crops except where the disease is prevalent in the seed at that time and the soil conditions are unfavourable for plant establishment. In these cases careful sorting on cutting would considerably reduce the risk.

Whether using whole or cut sets, poor stands are less likely if the seed is well sprouted and the soil conditions are favourable for rapid growth. Finally, it is of interest to record that the produce from plots planted with infected sets remained sound when carefully dug and stored in seed boxes until planting time the next season.

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Proceedings of the Association of Applied Biologists

Ordinary Meeting of the Association held on Friday, 9 November 1945, in the Imperial College of Science and Technology, London; the President, Dr C. B. Williams, in the Chair.

Discussion on factors controlling flowering

The following papers were read and discussed:

1. The need for research into the factors controlling flowering. By Prof. F. G. Gregory.
2. The event of flowering as viewed by a morphologist. By Prof. J. McLean Thompson.
3. Photoperiodic induction in *Tithonia speciosa*. By Prof. R. H. Stoughton.
4. Vernalization. By Miss O. N. Purvis.
5. Some external and correlative factors controlling flowering in the onion plant. By Dr O. V. S. Heath.

Ordinary Meeting of the Association held on Friday, 7 December 1945, in the Imperial College of Science and Technology, London; the President, Dr C. B. Williams, in the Chair.

Discussion on war-time problems of seed supply in Great Britain

The following papers were read and discussed:

1. General introduction. By Mr H. Hunter.
2. Britain's seed supply problems in war-time. By Mr L. E. Cook.
3. Seed-borne diseases. By Mr W. C. Moore.

Britain's seed supply problems in war-time

By L. E. Cook, *Seed Production Committee, National Institute of Agricultural Botany, Cambridge*

The seed needs of the U.K. in the pre-war period were met partly by home production and partly by imports. The seed trade had to look well ahead to provide against shortage and to alleviate the evils of surpluses by establishing export channels. In addition to the import and export trade, there was a considerable re-export trade. The value of the home seed industry before the war is not known, as there were no statistics kept, but the Board of Trade returns for 1938 show that imports were 576,699 cwt. valued at £1,052,611 and exports were 636,521 cwt. valued at £627,465 and re-exports were 54,190 cwt. valued at £116,092.

Seed requirements of cereals and potatoes are hardly within the scope of this paper, but it is worth recording that nearly one-tenth of the total cereal production, and nearly one-eighth of the total potato production is required for seed. Reference to cereals from the quality point of view will be made later in this paper.

The sources of our seed imports were many and widespread. Of the more interesting and important sources, New Zealand supplied us with cocksfoot, Chewings fescue, white clover and peas; Chile with red clover; Tanganyika with dwarf beans; U.S.A. with timothy, alsike, lucerne, smooth-stalked meadow grass (Kentucky bluegrass), fiorin (*Agrostis*) and vegetable and flower seeds; and Canada with alsike, timothy and lucerne. Nearer home, France, Holland, Denmark, Sweden, the Baltic states of Latvia and Lithuania, Germany, Poland, Hungary, Czechoslovakia, Rumania, Italy, Spain and Morocco in North Africa all contributed to our needs to a greater or lesser extent.

Home production had suffered severe competition during the previous 15 years, and in 1939 was in a very depressed condition. Low costs of production in such countries as Hungary, Jugoslavia, Rumania and North Africa had encouraged seed merchants to contract for vegetable and flower seeds in these

countries, not only for supplies of seed for use in this country, but also for a re-export trade. Grass-seed production in Denmark, and clover seed production in Poland, Hungary and Czechoslovakia had also forced prices to very low, and in some cases quite uneconomic, levels.

The seed farmers of this country were not organized and were not able to resist this competition. Whilst adequate supplies of seed were available, and the utmost freedom of trading was allowed, the Government did not find it necessary, nor were they called upon, to make any move. Indeed, the Government were only concerned with the enforcement of the Seeds Act 1920, which was enacted as a result of the experience of the 1914–18 war. During that war, the Official Seed Testing Station for England and Wales was established. The work of this station, now under the aegis of the National Institute of Agricultural Botany, has been a valuable contribution to the improvement of the quality of seed sold in this country. Annual reports of its work are published in the *Journal* of the N.I.A.B. Up to 1939 the number of seed samples tested annually had not exceeded 37,000 but since then, the number has risen, and for the past two seasons it has exceeded 74,000.

With the danger of war, the importance of ensuring a maintenance of our seed supply was realized, and in 1938 the Ministry of Agriculture re-established the Seeds Advisory Committee, who reviewed the position from time to time and advised the Ministry on all seed problems. This Committee met frequently until 1941, and still exists, but with the establishment of a Seeds Import Board, and a Seed Production Committee, its functions have lapsed. To-day the Seeds Import Board and the Seed Production Committee are both in full operation and the Ministry can turn to either the Board (S.I.B.) or the Committee (S.P.C.) for any advice that is required. These two bodies work in the closest harmony, and reference will be made to them again a little later.

Effect of war. Let us consider the effect of the war on our requirements. The ploughing up campaign and the 'dig for victory' campaign are remembered by everyone, but the relationship of these campaigns to our seed requirements may not be appreciated. The arable land was increased in 3 or 4 years by 6,000,000 acres. This called for an additional supply of nearly half a million tons of seed corn and for increased supplies of all other agricultural seeds. The increase in the number of allotments and the extension of vegetable production in gardens and market-gardens also meant a very big increase in vegetable seed requirements. Particular emphasis in the latter case was given to certain crops, such as onion and carrot, selected to help to feed people when imports of fresh vegetables were reduced or failed altogether.

Concurrently with these changes and increases in our requirements many of our usual sources of supply were disappearing. All European supplies stopped, and shipping from other sources became more difficult as the U-boat campaign was intensified. Shipments of seeds from U.S.A. and Canada were very vulnerable, and indeed a lot of seed was lost through ships being torpedoed. At one time, it looked as if supplies from New Zealand would cease, but fortunately the Japanese were not successful in cutting off this very distant part of the Empire from Great Britain.

Having now very briefly outlined the various stages on what may be termed the downgrade of our seed supply problems, we turn to 1941 and the following years, when there ensued and still ensues a period of intense activity and effort which has as its aim the assurance of adequate supplies of seed to meet the country's needs. The first stage was to ensure supplies for the immediate needs, and the next stage was to plan for the future, and finally to aim at the establishment of the seed industry on a firm basis, giving home producers every help and encouragement, planning production on sound lines and setting a standard of high quality production. By following out this plan, it is hoped that the seed industry—seed growers and seed merchants—will give better service to the consuming public, and at the same time establish the industry on sound economic lines.

Overseas supplies. As already indicated, the country's whole requirements cannot be produced at home, and it was therefore necessary to secure our supplies by development of all possible overseas sources as well as by the utmost expansion of home production. Before considering home production, and the problems involved, the overseas position should be considered. The supply of seeds from New Zealand never ceased and, in fact, this Dominion made great efforts to increase production of seeds. Their problem was a twofold one; it was necessary to increase home production in order to make good the deficiencies caused by the inability of the U.K. and other countries to maintain the normal supplies needed for the Dominion, and it was also their desire to grow more seeds for export to the U.K. to make good the deficiencies that were inevitable when our normal sources were no longer available. For export, production of cocksfoot, ryegrass, white clover, garden peas and rape were all extended materially, whilst for home use, mangold, swede, and turnip seed, and general vegetable seed raising was also undertaken. The climatic conditions of New Zealand are relatively favourable for seed production, and generally speaking, seeds produced there are suitable for conditions in the U.K. Only one instance has been recorded of seed from New Zealand failing to produce a satisfactory crop in this country,

that was seed of Pukekohe onion. This variety of onion seems to die off very early, with the result that the bulbs do not develop sufficiently and the yield is therefore very low.

Attempts have been, and indeed still are being, made to develop seed growing in other parts of the Empire—Australia, Malta, Cyprus, Kenya, Tanganyika. Dwarf beans from Tanganyika have been imported into the U.K. for some years, and are quite satisfactory, but from the other sources, supplies have been very limited. When attempting to establish seed production in a new area, it is essential to go slowly to start with. If seed is being produced from a biennial plant, it is 2 years before any seed can be available in this country, and then it must be tested out to make sure that the conditions under which it has been produced, length of daylight, temperature, humidity, etc., have not caused any deterioration in the plant. Everyone will agree that the evidence obtained in this way and in so short a time would be very slender, and that before entering into any large-scale commitments, a business man would require to see satisfactory results for at least two or three seasons. The lack of information as to the suitability of any particular place in the Empire as a potential source of supply has greatly hampered the trade and it would probably be well worth while devoting some time and money to carrying out some careful investigation into this subject.

The principal overseas source of supply at this time was the continent of North America. The seed trade in this country already had close connexions with the seed trade of both Canada and the U.S.A. When European sources failed, the trade immediately started to buy heavily from America, and they not only bought seed already grown and available, but they placed contracts for the growing of large quantities of seed. The seed growers and the trade of America made tremendous efforts to extend production, and were remarkably successful; a fact for which we must all be devoutly thankful. In the middle of 1941, however, a fresh problem arose, for we were informed that there was no money with which to pay for the seeds, and it was necessary to bring them under the scope of the Lease Lend Act so far as the U.S.A. was concerned. The country was able to pay for Canadian seeds for a little longer, but seeds from this source were eventually included in the 'Mutual Aid' programme.

It was in order to deal with this new situation that the Seeds Import Board was set up. The function of the Board was to plan requirements first from U.S.A. and then from Canada, and then to make all the necessary arrangements for their procurement, shipping, and distribution in this country. The Board meets in London every Monday to transact its business, but in addition, the members of the Board, nine in number, devote a great deal of their time to the preparation of information for the guidance by the Board of the Ministry. The work of the Board is carried out by an Executive Officer with a staff of about twenty people: a representative is stationed with the Combined Food Board in Washington.

With the advent of the Seeds Import Board, and the government handling of seeds, it quickly became clear that a closer planning and a more careful development of home seed production was essential. Early in 1942, the Ministry asked the N.I.A.B. if they would set up a committee to consider home seed production, and to advise on all problems relating thereto. This Committee was formed in February 1942, and consists of a number of members of the Council of the N.I.A.B., of the seed trade, and of the Farmers' Union. Mr (now Sir William) Gavin was appointed Chairman of the Committee, and I am the Executive Officer. Briefly the work of this Committee is to regulate, encourage and plan home seed production in relation to requirements, and to collaborate with the Seeds Import Board and the Ministry in maintaining the country's seed supply. This has necessitated the collection of a lot of statistical data, estimation of crops and planning of acreage requirements of various classes of seed. In addition to planning, the staff of the Committee has been available to help the technical staffs of county W.A.E.C.'s and individual growers where required.

Encouragement has been given to the establishment of county Seed Growers' Associations, and an increasing amount of attention is being given to the improvement of the technical aspects of seed production.

The N.F.U. have taken a great interest in this subject recently, and have now formed a specialist seeds branch at N.F.U. headquarters. As an outcome of the war, growers are now members of a central organization, and they, together with the seed merchants' organization, have collaborated in establishing seed-growing as a small, but important, and at present flourishing, section of the agricultural industry.

It is hoped that this seed industry will remain flourishing, for it is a valuable sideline to farmers, it is an all important part of agriculture, and it is a most fascinating and satisfying occupation.

Home production. Particularly interesting are the problems of the production of seed in this country under the abnormal conditions of 1939–44. It is proposed to treat that aspect of the work more fully, and to deal with the various seeds in groups: cereals, clovers and grasses grown by farmers as part of their normal farm practice, special strains of clovers and grasses, with very particular reference to the strains produced by the Welsh Plant Breeding Station, and, finally, root and vegetable seeds.

Cereals. The production of cereals for sowing is a very important part of corn growing. If the pre-war

statistical information published by the Ministry of Agriculture is examined it will be seen that the average yield per acre over a number of years is 18 cwt. for wheat, 16·4 cwt. for barley, and 16·2 cwt. for oats. The seeding rate varies for each kind of cereal, and according to the time of sowing, but on average it will be found that 10% of the total production of corn is needed for resowing. If it was possible for each farmer to use a proportion of his own corn for seed, this would be a very simple way of ensuring a continuous supply, but there are many reasons why this is not possible. Factors such as poor weather at harvest time, presence of loose or covered smut, weed or other impurities and mixture of varieties all suggest themselves in this connexion.

The increase in seed-corn acreage was greater in those areas where there had previously been very little, and consequently where there were few corn merchants accustomed to dealing in seed corn, and few farmers who were accustomed to taking the necessary precautions to grow corn of good seed quality. There was little that could be done to ensure large supplies of seed corn of first rate quality, but it is worth making some record of the steps taken to make the best of the situation.

(i) The price of seed corn was never controlled and thus merchants were able to pay, and growers to obtain, good prices for any parcels of corn particularly suitable for seed.

(ii) The N.I.A.B. started at the beginning of the war to build up small nucleus stocks of between 100–200 qr. each of the most suitable and popular varieties of wheat.

(iii) The N.I.A.B. was also requested to draw up a list of recommended varieties of winter wheats. The basis of consideration was the different types of land, and the various purposes for which the wheat is required. The very detailed and accurate trials, work carried out for over 20 years by the N.I.A.B. not only at Cambridge, but also at their regional sub-stations, provided sound scientific material to aid the committee in drawing up the list. Each trial provides for eight replications of the varieties under test for 3 years at several centres.

(iv) In a number of counties, schemes have been put into operation for the inspection and approval of corn crops for seed. Co-operation between Seed Growers' Associations and the technical staffs of the W.A.E.C.'s has provided the mechanism for these schemes. They are not all similar, particularly with regard to the authenticity of the crop varieties which are accepted for inspection. The Seed Production Committee, through its cereal seed sub-committee, and also the Council of the N.I.A.B. and the Agricultural Improvement Council have all given some thought to this subject, and it is hoped that a fully agreed scheme, acceptable both to the farmers, seed merchants and technical advisers, will be announced before long.

Clovers and grasses grown by farmers as part of their normal farm practice. Included in this group are ryegrasses, both perennial and Italian, red clover, sainfoin, trefoil, wild white clover and cocksfoot. The ryegrass crops for seed in Northern Ireland and Ayrshire, and to a lesser extent Aberdeenshire and the Fen areas round Ely, amount to approximately 100,000 acres, the majority being in Northern Ireland. Before the war, there was some export of this seed to the continent, but of course this ceased. However, with the big increase in the arable acreage, there was an increased home demand for seed. The acreage was actually increased to cope with the demand, and in 1942 a very large yield was obtained. The combination of large yield and increased acreage caused so much concern that arrangements were made for the Government to purchase the whole crop. This stabilized the position, and prevented a fall in price which might have been disastrous to future production. A restriction was placed on the production of seed of inferior quality and particularly of mixtures of perennial and Italian ryegrass, and also from seeds of older stands. Since these arrangements were made, consumption has increased still further, and supplies are to-day only just sufficient to meet requirements, and steps are being taken to try and stimulate further production.

Whilst mentioning ryegrass, a reference to 'blind seed' disease should be made. This disease is apparently becoming more prevalent, and results in a very low percentage of germination in many parcels of seed.

Red clover. Both broad red and late flowering types present a somewhat different problem. There is a far greater fluctuation in the amount of seed produced from year to year than with ryegrass. Ryegrass is harvested very much earlier, so that growers are not so much at the mercy of autumn weather as they are with reds. Efforts have been made to stimulate increased production of red clover, but frequently weather conditions have adversely affected the plants, either due to dry weather causing failure in establishment, or due to wet weather prevailing at harvest time.

Whilst our sources of supply of red clover are limited, volume of production is of first importance, but it is well not to overlook the importance of quality and of strains. There is not time to go into this subject in great detail, but it may be mentioned that the Welsh P.B.S. have introduced two strains of red clover, one of the extra late flowering type, known as S. 123 and the other an early or medium broad red, known as S. 151. In addition to these bred strains, attention has been directed to the importance of local, or indigenous strains, and in a

number of counties, local strains, particularly those which have been on the same farm, or group of farms for a number of years, have been sought out. The late R. D. Williams of the Welsh Plant Breeding Station carried out numerous investigations into the red clovers, and he recognized the importance of strain.

With the help of county seed production officers and others, strains which have a long association with a single farm or farming family, or village or group of villages or a particular ecological environment are being sought for. We do not know how far these strains will vary in productivity if they are removed to different conditions, or how far the seed from them will prove to be particularly adapted to the neighbourhood whence it originates.

In an endeavour to obtain more precise information, the Crop Improvement Branch of the N.I.A.B. are commencing replicated strain trials, and it may possibly be found that all the known strains have not sufficiently significant differences to justify a number of local schemes, but that it will be practicable and more valuable to combine the various local schemes into a national scheme, run on somewhat similar lines to the wild white clover inspection scheme. In the meantime, seed-production officers, or other technical officers of the county W.A.E.C.'s and the Seed Growers' Associations are co-operating in county schemes of inspections.

To encourage these schemes it has been agreed that seed from inspected and approved crops can be sold at a price premium of 3*d*. per lb. above the maximum prices which have been agreed between the seed trade and the N.F.U. and approved by the Ministry. Similar schemes are in operation for late flowering red clover. Schemes of inspection were in operation for red clover before the war in Essex, Montgomery, and Cornwall, but have now extended considerably. Taking broad red clover and late flowering red clover together, 503 acres in 1941, and 2633 acres in 1944 were inspected and this increase is in spite of the fact that there have been several very unfavourable seasons.

To maintain the country's normal requirements of red clover seed, and on the assumption of an average yield of 2 cwt. of cleaned seed per acre, it is necessary to aim at 50,000 acres or more each year.

The red clover crop is liable to reduction due to a number of causes. Bumble bees are necessary to ensure good pollination, and they seem to have been very scarce in the last few years. The honey bee is said to carry out some pollination, but it does not appear to be so effective. *Apion* weevil can damage the heads and seriously reduce the yield, and attempts are now being made to find some means of checking the attacks of this insect. *Sclerotinia* and stem eelworm both attack red clover, and cause some damage in most seasons.

Sainfoin is an important crop in Cambridge and Hertford, in Hampshire, Wiltshire, Dorset and in the Cotswolds. Inspection schemes on similar lines to the red clover schemes already outlined have been started. The types of common sainfoin in the three main districts are quite distinct, and in addition there is giant sainfoin.

Before the war, a considerable quantity of sainfoin was imported from France, but since the war we have had to be self-supporting. This has given an opportunity to investigate the types, and to sort out the best strains. In 1944, 872 acres were inspected under the local schemes. (Figures for 1945 are not yet available.) At the Cambridge Plant Breeding Station breeding investigations with sainfoins are being carried out by Mr J. L. Fyfe. In some years quite serious reductions in yield of sainfoin seed, especially giant sainfoin, are caused by gall midges (*Contarinia*). While some measure of control can be obtained by early hay cuts, there is a need for further investigation on this pest.

Trefoil. This is a popular seed crop in parts of East Anglia. Before the war a large quantity of trefoil seed was exported to Europe—France, Holland and Belgium. When this export trade ceased, there was a big drop in the demand for the seed, and production dropped rapidly. Unfavourable harvests in 1943 and 1944 caused a big rise in price, and as a result farmers grew an increased acreage for the 1945 harvest. With the end of the European War in the spring of 1945, there was an immediate prospect of a renewed export demand, and it chanced that favourable weather conditions occurred, so that the increased acreage has produced an exceptionally large crop, and the export demand has materialized, and we have been able to meet the demand for this seed. During the war years, damage has occurred to a number of seed crops of trefoil due to attacks by *Hypena variabilis*.

Wild white clover. Since 1930 there has been a scheme for the inspection and certification of pastures of wild white clover and during the war years there was a considerable revival of interest in this scheme.

After the 1914–18 war wild white clover rose to 25*s*. per lb. to the grower, but now a similar rise has not taken place. With increased supplies of New Zealand white, and with considerable supplies of Aberystwyth white S. 100, the demand for wild white may be somewhat restricted, as the other two clovers mentioned can certainly replace wild white in 2–3 year leys. The ploughing up campaign has caused a big reduction in the number of old pastures, but despite this, there is still a lively interest in the production of genuine wild white clover, and Kent growers are still obtaining a better price for their produce than growers in other districts.

Cocksfoot. The biggest development in herbage seed production has been in regard to cocksfoot.

This grass is a very valuable one, between 2000 and 2400 tons being used annually before the war. We relied almost entirely on imported seed. The principal source was Denmark, from which country between 1500 and 2000 tons were imported annually. New Zealand also supplied a quantity, and in addition there was a mere handful of growers producing seed in England, mainly from Danish or New Zealand stocks. All supplies except those from New Zealand ceased after the 1940 harvest, but production was developed in the U.S.A. and small supplies were also obtained from Chile.

In these circumstances, prices rose very rapidly, and this provided a stimulus to home production. In 1941 home production was less than 1000 acres, whilst by the 1945 harvest, seed was taken from 10,000 acres. This production includes the Welsh P.B.S. strains, the sowing of which increased from 481 acres in 1941 to 5447 acres in 1945. In 1945, over 3000 tons of seed were used, and it seems likely that even this high figure will be exceeded.

Cocksfoot is liable to severe damage from attack by cocksfoot moth. The moth lays its eggs at the flowering time, and the developing caterpillar eats the seed away, and then burrows out through the empty glumes, and finally enters the stem, where it pupates, the adult moth emerging the following spring. Work on this moth is being carried out by entomologists at Reading.

Welsh P.B.S. Strains. In 1941, 1497 acres were harvested for seed production of the following strains: cocksfoot S. 26, S. 37, S. 143; perennial ryegrass S. 23, S. 101; timothy S. 48, S. 51; red clover S. 123; white clover S. 100. (A further acreage planted in the spring of 1942, together with any acreage from crops being seeded for the 2nd or 3rd year, resulted in 2733 acres for seed harvest in 1942.) In the spring of 1942, it was decided to push for a considerable increase in acreage, and arrangements were made to earmark additional seed from the best crops inspected in 1941. By 1945, the acreage of these strains for harvest was 19,099 and a further 21,974 acres were sown, which with the 2nd and 3rd year crops should mean a production from 30,000 acres in 1946. The quantity of seed of the strains which should be available from this acreage is between 2500 and 3000 tons. The production of seed from these strains which are bred for their leafiness is not so easy as from commercial strains, and a new technique has been evolved by the seed production branch of the Welsh Plant Breeding Station. Very many farmers have undertaken to grow seed crops, but the general experience has been that a good deal of technical guidance is necessary. The cost of production of the stock seed by the Welsh P.B.S. is very considerable, and it is essential that the fullest use should be made of it. The present prices which are being paid to the grower are generally much higher than that paid for commercial seed, and it is to be hoped that before long, production technique will have improved sufficiently to enable the seed to be sold to farmers for use in mixtures at a much lower price than at present, whilst there is still a satisfactory return to the seed grower.

In the production of these seeds there have not arisen any serious problems of an entomological or mycological character other than those to which reference has already been made. Blind seed disease has been troublesome in the production of S. 24 perennial ryegrass; cocksfoot moth in all three strains, and *Apion* damage and shortage of bees have been a trouble in red clover seed production of S. 151 and S. 123.

Root and vegetable seeds. With the exception of sugar-beet seed and rape seed, this country was before the war self-supporting in root and fodder seeds, and there was also an export trade.

During the war we have developed to a stage of self-sufficiency in sugar beet, and in most seasons, sufficient rape is also produced.

Before the war only about 50% of the sugar-beet seed was home-grown, and for most of that, we relied on continental sugar-beet seed firms for stock seed. During the war, we have maintained our own supply. Whether English firms can maintain the same standard of quality of sugar-beet seed as that previously obtained from abroad, cannot yet be determined but there has been no drop in yield, either of roots per acre or of sugar per ton of roots. There are three problems of sugar-beet seed production, which are of biological interest. The troublesome attacks of aphis, the incidence of downy mildew and the disease known as virus yellows. Considerable success has been obtained in the control of aphis by nicotine gas, but this is a very specialized form of treatment, for which growers have to rely on specialized firms with appropriate equipment. Virus yellows is perhaps one of the worst troubles and Dr Hull of the Midland Agricultural College is studying its incidence and control, and it is hoped that he will be able to give material help. Eelworm is also a source of damage and loss, and seed crops must not be grown in eelworm infected areas.

Mention should also be made of another very troublesome pest—the pollen beetle, which does such severe damage to brassica seed crops, particularly swede. This subject is being studied at Wye.

Vegetable seed production. Over 50% of the vegetable seeds produced in the U.K. are grown in Essex. Here, in the area round Kelvedon, Coggeshall, and Marks Tey, are found a combination of climatic and soil conditions which are particularly adapted to this form of production. Unfortunately, no statistics are available on pre-war production, but

it is said that there has been a big increase in the production of many kinds of seed. In 1945, between 25,000 and 30,000 acres were devoted to vegetable seed production. An account of vegetable seed production is given in a publication by the Imperial Agricultural Bureaux (Miscellaneous Publication No. 5).

In technique, the extended use of tripods for harvesting peas, the increased use of drying plants for many classes of seed, the control of pests by spraying and dusting, particularly on brassica crops, but to a smaller extent on peas, beans, beet and mangolds, and the development of an acid extraction method for the removal of tomato seeds from the fruit, may be mentioned as examples of progress.

Orderliness has been introduced into the vegetable seed growing industry in a number of ways. The Growing of Seed Crops (Control) Order, introduced on the advice of the Seed Production Committee requires that no grower shall grow any seed crops of the principal kinds except after making a contract with a licensed seed vendor before a fixed date. The merchants are required to notify contracts to the Seed Production Committee. Before the fixed date for contracts, the Seed Production Committee invite all licensed vendors to submit a statement of their acreage proposals for each kind of seed and the committee considers the proposed acreage in relation to the estimated requirements, and firms are advised whether the total acreage is considered too great or too small. The trade and the growers appear to have found this forward planning of considerable assistance.

A zoning scheme has also been put into operation in a number of counties to assist merchants and growers to secure satisfactory isolation of crops from any other crops with which they might be cross-pollinated. Though this scheme has been run on a voluntary basis, it has achieved a remarkable degree of success in most areas.

Before concluding a brief reference should be made to the financial aspects of the seed industry. With a few exceptions, growers' prices have been agreed between seed growers and wholesale seedsmen. Wholesale and retail prices have been considered by representatives of the trade interests concerned, who have then submitted the proposals to the Ministry. The Ministry refer them to a Prices Sub-Committee of the Seed Production Committee, who consider them with representatives of the bodies who have submitted them. In many cases, the prices submitted have been modified by the Prices Sub-Committee, for the trade representatives are usually authorized to deal with any reasonable suggestions. When the Prices Sub-Committee are satisfied, they recommend the suggested prices for approval by the Ministry. No price regulations have been made, and both growers and merchants have, with very few exceptions indeed, accepted these recommended and approved prices.

The seed industry has received tremendous stimulus as a result of the increased calls that have been made on it. It is a section of farming which is peculiarly suited to certain parts of this country, and the production of seeds of high quality should always be maintained. Co-operation between seed growers and seed merchants has developed enormously during the past few years, and the future development of the industry depends to a large extent on the continuance of this co-operation. In addition, still further help will be needed from biologists—the ecologists, the geneticists, the plant breeders, the entomologists and the mycologists—who can all contribute in their respective fields.

Seed-borne diseases

By W. C. Moore, *Plant Pathology Laboratory, Milton Road, Harpenden, Herts*

I can claim no special knowledge of seed-borne diseases apart from a lengthy experience of examining seeds for health certification prior to export from this country. However, that experience has certainly been more than sufficient to impress me with the importance of producing healthy seed and with the risks that may be attached to the uncontrolled movement of diseased seed. War-time problems of seed supply undoubtedly increased the risks, but there is very little reliable evidence about the precise effect of the abnormal conditions, and with your indulgence I would prefer, in the time available, to consider some of the wider aspects of the subject.

First, you might like to know a little about the health examination of seeds carried out at the Ministry of Agriculture's Plant Pathology Laboratory at Harpenden. Before the war there was no sort of international standard procedure. Some countries, including our own, permitted unrestricted entry of seeds: others demanded a certificate of health for certain specified seeds or for all seeds, and a few had special regulations. South American countries, for example, required certificates of health for all seeds, while the United States of America asked for certificates only for seeds of trees, shrubs and sweet peas. The Dutch East Indies, on the other hand, supplied a long free list but required certification for all seeds not on that list.

Seedsmen in this country who receive orders from abroad naturally have to comply with the regulations of the importing country. If health certificates are demanded, the seedsman gets into touch with the Ministry of Agriculture and Fisheries, who arrange for an Inspector to take samples of the seeds to be exported. The Inspector then sends the samples to Harpenden for examination. Occasionally the actual seeds to be exported are sent, but this is a nuisance both to the seedsman and ourselves because it involves much unpacking and re-packing. The usual method is for the Inspector to draw samples from stocks of the varieties ordered. If the samples are passed, the stocks from which they come can be drawn upon for subsequent orders without re-examination: if a sample is turned down, the whole stock from which it is drawn is automatically turned down. A report on the results of the examination is sent from Harpenden to the appropriate branch of the Ministry, who either inform the seedsman that such and such seeds cannot be passed, or issue to him a certificate stating that the seeds, or a representative sample of them, were thoroughly examined at the Ministry's Plant Pathology Laboratory, Harpenden, on such and such a date, and 'were found or believed to be free from injurious plant diseases and dangerous insect pests'.

To give an idea of the extent of the work I may say that between 1925 and 1943 nearly 30,000 seed samples were examined. The numbers steadily rose from about 500 a year at the beginning of the period to over 4000 in 1939 and then dropped very rapidly. The great majority were flower seeds and the rest mainly vegetables, with a few cereals. Of the 30,000 samples 650, or about 2%, were rejected on the grounds that they carried parasitic organisms. Most of the samples rejected consisted of peas, celery, and parsley: 27% of the pea samples submitted have been rejected because they were infected with species of *Ascochyta* or showed the internal symptoms of Marsh Spot; 42% of all celery samples examined have been infected with *Septoria*, and 31% of the parsley samples with *S. Petroselini* Desm. Other rejections comprised a very miscellaneous lot, the most frequent items being Dwarf Bean seeds affected with halo blight (*Pseudomonas phaseolicola* (Burkh.) Dowson) or less frequently anthracnose (*Colletotrichum Lindemuthianum* (Sacc. & Magn.) Bri. & Cav.); sunflower seeds mixed with or carrying the sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary; and beet seeds bearing rust spores (*Uromyces Betae* (Pers.) Lév.) or pycnidia of *Phoma Betae* Frank.

One of the objects behind seed inspection of this nature is to prevent the introduction of diseases *new* to the importing countries. To what extent this is achieved is perhaps a matter of doubt: every care is taken to carry out the regulations laid down by importing countries, but I would not claim more than that we do to a large extent prevent the more easily recognized diseases from being exported with our seeds.

During the war the numbers of seed samples received for health certification dropped almost to nothing. It was necessary, however, to be on the watch for new diseases that might have come into this country on or in the seed it was necessary to import. Several apparently new diseases are reported most years and the last few years have not been exceptions, but it is extremely difficult to trace the origin of these diseases. It is usually some years before they become sufficiently severe to attract the attention of the grower, by which time it is almost impossible to find out how or where they started. To mention but one example. Bacterial canker of tomato (*Corynebacterium michiganense* (E.F.Sm.) Jensen) is primarily a seed-borne disease which was not recognized in Britain until 1942, when two suspected cases were seen under glass in Sussex (Ware & Glasscock, 1944). The following year ten further outbreaks were confirmed, mostly in outdoor crops, and in six of these the same variety, derived directly or indirectly from the same source of seed, was affected. Inquiry showed that the seed had been imported from a country where bacterial canker is well known. Official action was taken to prevent further distribution of the seed and to deal with individual outbreaks, and the measures adopted were evidently effective, for the disease has not reappeared on any of the infected nurseries. Several fresh cases were discovered in 1944 and 1945 and by November 1945 there were thirty-one known outbreaks in England, mainly in the south-east and the Isle of Wight. The disease does not appear to have done much damage under glass, but in ten of the twenty-six outbreaks confirmed in the open the loss was moderate to severe. For example, in one nursery about one-quarter of 8000 plants were killed by it and many others were also affected; at least 30% of a 4-acre crop was affected in another nursery; and in a third practically every plant of a ½-acre crop was attacked. There can be little doubt that bacterial canker was brought into England with imported seed during the war, but it does not follow that all the outbreaks began in this way, or indeed that the disease was not present here to some extent under glass before the war. A few other diseases that might have been introduced with imported seed could be mentioned, but the evidence is by no means conclusive. It may be, too, that there are still some not yet recognized, but on the whole we seem to have escaped lightly.

A justifiable conclusion from these facts is that seed inspection and rejection does not appear to be a particularly effective method by itself of dealing with the problem of seed-borne diseases. This is not surprising in view of the large number of seed-borne

parasites and the varied ways in which they can be carried in the seed, some of them impossible to detect merely by examining or even germinating the seed.

As to numbers it is possible to give two sets of figures, though they will doubtless be added to very considerably when the subject is given the attention it deserves. The bibliography of seed-borne parasites compiled from many sources by C. R. Orton (1931) listed approximately 250 different organisms on nearly 150 cultivated plants. The organisms were mainly fungi but included about fifty species of bacteria, a few viruses and one or two eelworms. A more recent and more select list of seed-borne fungus diseases has been compiled by Neergaard (1940), who included only forty-two vegetable and ornamental plants, on the seeds of which he listed just over 100 pathogens, including ten Phycomycetes, ten Ascomycetes and seven Basidiomycetes. The rest were Fungi Imperfecti.

Representatives of all the chief groups of fungi are seed-borne in one way or another. Some fungi, of which the one that causes bunt of wheat is perhaps the best known, are carried mechanically in the form of spores on the surface of the seed. Parasitic species of *Alternaria*, *Helminthosporium*, rusts and other fungi are also carried in this way and they can usually be detected by steeping the seed in water and examining the washings under the microscope. Others are transmitted solely as mycelium, very difficult to detect, inside the seed. The best known of these are the loose smuts of wheat and barley. Species of *Septoria* can frequently be detected under a binocular microscope in the form of pycnidia embedded in the testa and other tissues of celery, parsley, lettuce and other seeds. The same may be true of *Ascochyta*, *Phoma* and other Sphaeropsidales but one of the commonest of these, *Ascochyta Pisi* Lib., is very difficult to detect in the dry seed, though pycnidia are generally formed in abundance on infected seed kept for a week or so in moist sand. Again, the sclerotia or resting bodies of ergot (*Claviceps purpurea* (Fr.) Tul.) and certain other fungi, notably species of *Sclerotinia* and *Botrytis*, are often found mixed with seed of grasses, cereals, sunflower, clover, sainfoin and other plants. This is not and cannot be a hard and fast grouping. Some of the smut fungi appear to be carried as mycelium in the seed coat as well as in the form of spores on the seed surface: fungi that form pycnidia in or on the seed are also transmitted in the form of internal mycelium: and sclerotia may be found in as well as among the seed. Many bacterial diseases are also seed transmitted; indeed, Orton (1931) was of the opinion that every bacterial disease should be considered as potentially seed-borne. The bacteria may be present on or in the seed, as in bacterial canker of tomato and black rot of crucifers. There are differences of opinion about the extent to which virus diseases are transmitted through the seed, but it is generally accepted that the wide distribution and severity of bean and lettuce mosaics are largely due to the virus being carried in this manner.

Time will not permit more than an indication of a few ways in which the problems of seed-borne disease might be tackled. The subject has been given far too little attention in the past both by seed-testing laboratories and by plant pathologists, and perhaps the best hope for the future lies in a close partnership between them on the one side, and collaboration with seed producers and seed merchants on the other. One of the most important things is more information: more information on the extent to which diseases are seed-transmitted, especially in vegetable and flower crops, more information about the morphology and biology of seed infection, about the behaviour of the parasites during seed dormancy, and about the factors underlying the often baffling occurrence and appearance of a disease in crops raised from infected seed. The excellent monograph recently published by Neergaard (1945), in which are embodied the results of careful mycological studies carried out in a commercial seed establishment in Denmark, provides a notable example of the opportunities available.

There is, too, an enormous field open for improvements in methods of seed production, and for field inspection and certification of seed crops. The very existence of diseased seed stocks carries with it the virtual certainty that yields were not what they might have been, and it is well known that *Botrytis* alone can ruin many kinds of seed crops during the weeks immediately preceding harvest. Other methods that suggest themselves are seed disinfection or seed treatment, methods of rapidly testing seeds for disease diagnosis, and international agreements regarding seed import and export. Nor must it be forgotten that careful study of a disease may provide information that will enable one to select regions or districts in which seed crops can be grown with little risk of harvesting infected seed. When Hickman (1941) examined commercial pea-seed samples grown in England or abroad, he found that twenty-six out of twenty-nine samples grown in England were infected with *Ascochyta*, sometimes to the extent of nearly 50%, whereas out of thirty-one samples of the same varieties grown in Hungary, Morocco and New Zealand only nine were infected, and of these only two showed more than 5% of the seeds attacked. Similar differences have also been reported between pea seed grown in the semi-arid western parts and in the wetter eastern areas of the United States of America (Jones, 1927).

Seed disinfection and seed treatment may still be more or less in their infancy. Thanks largely to the efforts of commercial firms and research workers, backed by official support and publicity, the organo-

mercury dusts provided a simple and highly effective method of dealing with some of the seed-borne diseases of cereals during the war. Certain flax diseases were dealt with in the same manner, though the dust used was a different one and contained no mercury. Newer organic dusts have recently been widely used on vegetable seed in North America, and there is scope for extending these trials as well as experimenting with fumigants and hot-air treatment.

Health certification of seed crops in the field would seem at present to provide the only solution for obtaining seed stocks free from virus diseases and from those bacterial and fungus diseases which are carried internally in the seed. Nevertheless, field inspection has its limitations, for there may be ample opportunity for infection by one parasite or another to take place during harvesting and threshing, or in storage. In short, these different avenues of approach must be regarded not as alternatives, but as complementary or supplementary to one another. Sometimes one, sometimes another may be sufficient, but the ideal might well be field inspection and certification, followed by scientific harvesting and handling and if necessary seed treatment, and finally by seed testing that places just as much importance on freedom from disease as on purity and vitality.

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Proceedings of the Association of Applied Biologists

Annual General Meeting of the Association held on Friday, 22 February 1946, in the Metallurgical Lecture Theatre of the Imperial College of Science and Technology, London; the President, Dr C. B. Williams, in the Chair. After the formal business there was held a

Discussion of the official scheme for the approval of proprietary products for the control of plant pests and diseases.

The following papers were read:

1. Past attempts to establish an approval scheme. By Dr H. Martin.
2. The structure and operation of the official approval scheme. By Dr J. T. Martin.
3. A manufacturer's comments on the approval scheme. By Dr J. R. Booer.
4. The approval scheme as seen by a specialist advisory officer. By Dr W. A. R. Dillon Weston.
5. The grower's impressions of the approval scheme. By Mr O. G. Dorey.

Past attempts to establish a scheme for the official approval of proprietary insecticides and fungicides

By Hubert Martin, *Long Ashton Research Station, Bristol*

The aim of any scheme of approval of insecticides and fungicides must be the provision of a standard description of an insecticide or fungicide suitable for official recognition and with a common meaning to those who use it. In some cases, bluestone for instance, the problem seems easy, for bluestone is a simple chemical, the pentahydrate of cupric sulphate. In other cases difficulties are obvious, as in lime sulphur, a product of complex chemical composition obtained by boiling sulphur with milk of lime. Yet lime sulphur is so widely used as a fungicide that it is important to ensure that the meanings given to the

name by grower, adviser and maker are synonymous and that the grower can tell which of the many commercial brands of lime sulphur answers this common description or specification.

In putting this idea into practice, difficulties at once pile up. To specify that the bluestone should be of the highest attainable purity would involve the purchaser in unnecessary expense if it is known that the usual impurities do not detract from the performance of the product as a fungicide. The standard is accordingly fixed to include the normal commercial grades of crystalline copper sulphate, thus becoming a minimum specification. In other cases it may be necessary to stipulate that the content of concomitant material which detracts from biological efficiency shall not exceed a certain maximum figure. A fair prescription of these limiting figures can only follow a multilateral agreement of the official, the trade and the grower's interests.

In this country the first step in the formulation of officially approved specifications for insecticides and fungicides followed representations to the Board of Agriculture made by the Chamber of Horticulture, an organization primarily of the horticultural trade established in 1918 (see *The Gardeners' Chronicle*, 1918, **64**, 170, 228). The outcome of these negotiations in which the Government Chemist and an important section of manufacturers took part, was the drafting of a Bill for the regulation of the trade in certain of the chemicals most generally in use for the control of pests. In 1921, because of Cabinet instructions on national economy, the introduction of the Bill to Parliament was postponed but some of its more important provisions were published in the Ministry's Journal (*J. Minist. Agric.* 1921, **28**, 628) in the hope that manufacturers would be prepared to meet the terms of the Bill without previous legislation. Purchasers of the materials specified were advised to stipulate that the articles supplied should conform with conditions laid down.

These hopes were to some degree realized and, in due course, the question of the revision and extension of the original provisions arose. Meanwhile, the place of the Chamber of Horticulture seems to have been taken by the National Farmers' Union, and the Association of British Insecticide Manufacturers (A.B.I.M.) now served for the representation of the manufacturers. The latter organization undertook this work which in July 1934, was published as the Ministry's Bulletin No. 82. The additions made to the original list were few but an important advance was made in the publication of agreed methods of analysis on which the specifications were based. The analytical chemist will ask why these methods were omitted from the original publication in 1921 for it is indeed difficult to see how the 1921 proposals could have served for purposes of legislation without official methods of analysis. But even in their earlier recommendation as published in the Ministry's Bulletin, No. 363 (Oct. 1921; revised Dec. 1928) the one analytical method included in the original, that for liver of sulphur, was omitted together with the specification. The value of agreed analytical methods to the trade needs no stressing but the grower does not seem to have appreciated that the standard analytical method provides for the checking of the manufacturer's guarantee of conformance should this checking be necessary in cases of dispute or dissatisfaction.

At their winter meeting in 1933 the Conference of Advisory Entomologists discussed the problem of the proprietary insecticide, in particular, the tar-oil and petroleum-oil preparations which by then had revolutionized the fruit growers' winter spray programme. A committee was appointed with wide terms of reference and, at its meeting on 21 March 1934, I was rash enough to suggest that these materials could be covered by specifications. Naturally, I was told to produce the specifications and the evidence on which they were based, a task completed in a lengthy memorandum which this Association permitted to be published in its *Annals*. My suggestions were kindly but critically received and in due course a joint committee of the Ministry of Agriculture and the Association of British Insecticide Manufacturers was formed to consider the preparation of specifications and methods of analysis for the tar-oil winter washes. Their report, after approval by the trade and the Government Chemist, was published in January 1941, as the Ministry's Bulletin No. 122. Similar specifications for the petroleum oil washes followed in due course and the deliberations on preparations containing both tar and petroleum oil as actual ingredients are now approaching completion.

By 1934 it was evident that the home-made spray and simple types of proprietary catered for in Bulletin No. 82 were quickly being replaced by compounded proprietaries such as the dispersible copper-containing powders and the organo-mercury seed disinfectants. To deal with such products by the specification method would clearly take many years and it is not surprising that alternate ways of obtaining official approval were sought and discussed, in particular, the possibility of replacing the physico-chemical analytical methods by biological methods. At the Third Imperial Mycological Conference in September 1934 a resolution was passed directing attention to the need for fundamental research into methods for the biological standardization of fungicides and insecticides. By 1936 the Ministry began active enquiries into the feasibility of initiating and sponsoring a more determined attack on the problems of biological testing, gathering information on the extent to which known and proved methods of test would cover the field and discussing

matters concerning the personnel and facilities required for an expansion of this work.

The outbreak of the 1939–45 War brought rapid developments. The Plant Pathology Committee of the British Mycological Society appointed a sub-committee to report on systems of recommending the use of proprietary insecticides and fungicides. Their report, dated 27 May 1940, suggested a scheme of registration with a 'white list' of approved proprietaries as its end point. Our Association was at this stage invited to co-operate in the extension and development of the scheme and a joint sub-committee was set up for this purpose. From informal discussion it was learnt that the prospect of early official acceptance would be strengthened, firstly, by the avoidance of legislation, i.e. the scheme should be voluntary and, secondly, if the scheme solved certain difficulties met in the earlier officially sponsored discussions mentioned above. Although it was not expected that the whole range of proprietaries could be dealt with at once, it had to be shown that the scheme was able to meet all difficulties likely to be met in its extension to the full range. These difficulties centred round two issues: (*a*) the safeguarding of the manufacturer wishing to keep the composition of his product secret from unwitting disclosure; (*b*) the machinery required for the extension of approval to new types of product.

The report of the joint committee, after approval by the parent bodies, suggested a plan following closely that previously accepted by the British Mycological Society but extended to operative details and three appendices dealing with specific examples of the analytical and biological tests required for ovicides, protective fungicides and seed disinfectants. Moreover, the possibility was envisaged that the scheme might extend to products for domestic and industrial use which are beyond the interests of the Ministry of Agriculture. Briefly the scheme was to be under the control of an administrative committee representative of the several interested parties, with a Secretary or Code Officer as the executive officer. The identity of each product submitted was to be hidden by a series of code numbers but certain particulars and assurances were required of the manufacturer based on schedules drafted for each broad group of product by Coding sub-committees. Similarly, for each group, a schedule of tests of biological efficiency, stability and foolproofness was to be drafted by Testing sub-committees. The physico-chemical tests involved were to be carried out at a central laboratory and the field tests by special assistants with appropriate facilities at existing research and advisory centres. The results of these various tests, after collation by the Code Officer, were to be reviewed by appropriate Approval sub-committees who would report to the Administrative Committee where, for the first time, the identity of the product, if approved, would be revealed.

The scheme, approved as a basis for discussion by the Ministry in January 1941, was submitted to the A.B.I.M. who, after rapid but full consideration, reported that the general principles of testing and registration were unacceptable to the Association. Further the Association considered that the necessity for such a scheme had not been established and that if it were the scheme should be put forward by the Ministry. The Ministry accordingly handled subsequent negotiations and in December 1941, a simplified plan was submitted to the Association. Simplification was secured by abandoning the idea of officially sponsored tests, both analytical and biological, by refusing to admit products of the wholly secret type and by limiting the scheme to products for use against plant pests and diseases. After agreed modifications the Association expressed their willingness to co-operate and the scheme was put into operation in October 1942.

The structure and operation of the official approval scheme

By J. T. Martin, *Ministry of Agriculture and Fisheries Plant Pathology Laboratory, Harpenden, Herts*

When asked by the Council of the Association to give an account of the official scheme for the approval of proprietary products for the control of plant pests and diseases, I accepted the invitation since it clearly was desirable, if the discussion by the Association of the scheme was to be of value, that its structure, what it attempts to do and what it does not do should be fully understood.

In some countries attempts have been made to give guidance on the purchase of proprietary insecticides and fungicides by the official testing of products to ensure that they reach satisfactory standards, by legislative control or by both. Such methods are inherently costly, are limited in application, and take considerable time to put into effect. If an approval scheme was to play any part in increasing the supplies of foodstuffs needed under war conditions in this country, it was essential that the many different groups of insecticides and fungicides should be dealt with as speedily as possible. The Ministry of Agriculture and Fisheries and the Department of Agriculture for Scotland, with the co-operation and

agreement of the Association of British Insecticide Manufacturers as the representative body of the insecticide and fungicide trade in this country, therefore put into operation in October 1942 a purely voluntary scheme for the official approval of insecticides and fungicides sold under brand names.

The structure of the scheme

The main provisions of the scheme

The scheme has received the strong support of the Agricultural Improvement Council. Its main object is to secure the official approval of proprietary materials so as to enable anyone professionally concerned with giving advice on the control of plant pests and diseases to recommend suitable ranges of products by their proprietary names. The scheme is confined to preparations generally available for purchase in Great Britain for the control of pests and diseases of growing crops; it is not concerned, for example, with products used in veterinary work or with products for the protection of stored crops. Participation in the scheme is open to any manufacturer or his authorized agent in Great Britain. It is also open to the authorized agent resident in Great Britain of a manufacturer overseas; in such a case the agent in Great Britain is required to accept responsibility for compliance with the conditions laid down by the scheme and, as with the manufacturer in this country, only he may make use of any approval granted.

An approved product shows on its container the approval mark of the Ministry and the Department. The mark consists of a diamond-shaped design bearing the words 'Ministry of Agriculture and Fisheries' and 'Department of Agriculture for Scotland', and enclosing a crown. Beneath this is a phrase indicating the guarantee, by the manufacturer, that the product conforms to a standard approved by the Ministry and the Department for the purposes indicated on the container, and referring to the inclusion of the product in the approved list. The mark shows the group and product references for purpose of identification and the whole is enclosed in a rectangular panel.

Care was taken in designing the mark to make it as distinctive as possible. The distinctive nature of the mark enables a customer entering a shop to see at a glance whether a product has been approved. Certain conditions concerning the use of the mark have been laid down. It may be used only on the containers of an approved product and then only on containers bearing the proprietary name under which the product has been approved. It may not, for example, be used in advertisements or on advertising leaflets issued in connexion with products, although it may be shown on a container pictured in an advertisement. Approval is granted only in respect of conditions in Great Britain; the mark should not, therefore, be used on the containers of approved products sent overseas. If manufacturers wish, the use of a phrase on such containers indicating the approval of the product by the Ministry and the Department for certain uses in Great Britain is permissible. For the purpose of the scheme, the Channel Isles and the Isle of Man are regarded as parts of Great Britain.

As indicated earlier, an application for the official approval of a product on the part of a manufacturer is entirely voluntary. It is of interest to note in this connexion that, as far as the groups of products hitherto considered permit, all the leading manufacturers and many smaller firms have taken advantage of the scheme. If a product is not submitted for approval, or if a product is submitted and approval is not granted, the withdrawal of the product from the market is not required. Any product whose composition the maker wishes to keep wholly secret from the public (even although he may be willing, in an application for approval, to disclose some details in confidence) is not eligible for consideration under the scheme.

Among other undertakings, the manufacturer guarantees that after approval, the composition of the product will not be changed without the agreement of the Ministry and the Department. All applications for approval are strictly confidential, and since a product may not be approved, not because of inefficiency, but merely because of lack of information concerning it, the fact that a particular product has failed to secure approval is not disclosed.

The scheme provides for the periodic issue of lists of approved products for the use of consumers and advisory officers. At approximately twelve-monthly intervals, leaflets giving the full list of approved products are published; additions to the list made between successive issues of the leaflet are officially notified in *Agriculture*. The leaflets are sent to all advisory officers in Great Britain, to the War Agricultural Executive Committees and others interested, and to many individuals and organizations who have applied for them. When giving assistance on pest or disease control, the adviser is required to give the names of all the approved products suitable for the purpose in view.

The name of any product may be withdrawn from the approved list if evidence becomes available which shows that the provisions of the scheme have not been complied with. Any case of complaint following the use of an approved product can be submitted for investigation. Failure to obtain adequate control following the use of an approved product may, however, be due to a number of reasons unconnected with the product as such, for example, adverse weather conditions following application or the use

of inefficient methods. Before any action is taken, therefore, evidence is required from the person making the complaint that the product itself is at fault.

The administration of the scheme

The Ministry and the Department are advised on the conduct of the scheme by an Advisory Committee and a Joint Panel.

The Advisory Committee consists of independent scientists drawn principally from Government Departments and from Research Stations, and nominated by the Minister of Agriculture and the Secretary of State for Scotland in consultation with the Agricultural Research Council. Its constitution is as follows: Prof. J. W. Munro, M.A., D.Sc. (*Chairman*); C. E. Foister, B.A., Ph.D.; C. T. Gimingham, O.B.E., B.Sc., F.R.I.C.; A. J. Holden, B.Sc., F.R.I.C.; H. Martin, D.Sc., A.R.C.S., F.R.I.C.; W. C. Moore, M.A.; D. D. Pratt, M.A., Ph.D.; H. Shaw, M.Sc., Ph.D.; J. T. Martin, D.Sc., F.R.I.C. (*Secretary*).

The Advisory Committee forms the official body responsible for the examination of applications and for the recommendation, to the Minister and the Secretary of State, of individual products for approval. The Committee has power to consult persons outside its membership and to appoint assessors to deal with specific problems; its proceedings are, however, strictly private and confidential.

It should be emphasized that no member of the Advisory Committee is connected with any firm or firms compounding or marketing proprietary products; perfectly unbiased examination of applications is secured and there is no danger of the formula for a product being revealed to a competitive manufacturer.

The Joint Panel consists of the members of the Advisory Committee and members nominated by the Association of British Insecticide Manufacturers, by the Agricultural Research Council, and by the Government Chemist. The Chairman of the Advisory Committee is also Chairman of the Joint Panel. The functions of the Joint Panel are to draw up standards and to lay down principles for the guidance of the Advisory Committee in its consideration of applications for approval, and in general, to deal with the wider aspects of the scheme. The Joint Panel also has power to consult persons outside its membership on specific problems.

The basis of approval of products

No provision for the testing of products as a basis for approval is made under the scheme. Instead, products are approved under two categories as follows:

(*a*) *Approval on the basis of conformity to specification.* A product which is guaranteed by the applicant to conform to a specification accepted by the Ministry, the Association of British Insecticide Manufacturers and the Government Chemist, and by the Joint Panel as suitable for the purposes of the scheme, is recommended by the Advisory Committee for approval without specific evidence of efficiency, provided the claims and recommendations for use shown on the labels are approved.

An explanation of the reason for this procedure is perhaps necessary. The specifications are chemical specifications, and in preparing these attention is given to all the characteristics concerned with efficiency. As an example, the specification for miscible tar oil winter washes may be quoted. The specification requires that a wash shall contain not less than a stated percentage of total oil, and that of this not less than a certain amount shall consist of a particular fraction of tar oil, closely defined by distillation range. The phytotoxicity aspect is covered by the specification requiring that the wash shall contain not more than a certain percentage of phenols, and the physical condition of both the undiluted and diluted wash by requirements that the materials shall pass certain tests for stability. Prescribed methods of analysis are drawn up for use with the specification.

The specification is based upon the knowledge, gained from past experience, of the characteristics which the wash must show for efficiency when used at the normal rates of dilution, and is also based upon the assumption that it will be reasonably efficiently applied. The requirements shown by the specification are thus linked with the directions for use; the approval of claims and recommendations appearing in the labels of products clearly was desirable, and provision for this was included in the scheme.

The granting of approval on the basis of conformity to specification forms the logical sequence to an earlier agreement on the part of the manufacturers to conform to official specifications in the compounding of their products.

For the benefit of those not familiar with the term and to avoid a possible misunderstanding, the word 'conforms' in the phrase 'conforms to specification', as used by us, means complies with, or reaches the standard set by, the specification. It does not mean, for example, that all the products in a particular group have been raised, or reduced, to a common standard of composition. There will be, therefore, in any group dealt with by specification, products showing differing levels of efficiency, but all reaching the standard laid down. No attempt is made under the scheme to place approved products in a particular group in order of merit; to do this would be undesirable and to a large extent, impracticable.

(*b*) *The basis of approval of other products.* The preparation of specifications with accompanying methods of analysis is, in some cases, a difficult and time-consuming matter. In addition, there was, at

the time of the commencement of the scheme, a number of important groups of materials for which specifications, in the existing state of knowledge, could not be prepared, and it was desirable that these groups should be dealt with at an early date. A second basis of approval was therefore included in the scheme. In this category, products became eligible for approval on the basis of evidence of efficiency in use, which could be supplied by the manufacturers or could be obtained from the advisory officers, research stations or any other suitable source. In addition, schedules of requirements were drawn up by the Joint Panel for products considered under this heading; the information required was reduced to the minimum necessary to characterize the products and to guard against possible inefficiency through factors such as, for example, loss of activity in storage. As with products dealt with by specification, the labels of products dealt with in this way also needed to be approved.

With products in both categories, the approval granted thus relates to the product as such and to the claims and recommendations for use. A commercial or amateur grower should therefore have confidence that a proprietary product bearing the official approval mark, if reasonably efficiently applied, would be satisfactory when used for the purposes and according to the recommendations for dilution shown on its label.

The approval of products on the basis of conformity to specification is, without doubt, the more expeditious and satisfactory of the two methods of dealing with products, and the consideration of as many groups as possible on the basis of conformity to specification must remain the aim of the approval scheme.

The schedules of requirements drawn up in connexion with the second category of products have proved of great value in indicating the gaps in our knowledge which must be filled before specifications can be prepared.

The approval of labels

A few points concerning the consideration of labels may be of interest. Claims of an extravagant or erroneous nature, of the control of pests or diseases for which a particular product clearly is unsuitable, or which imply that particular products are superior to others, have not been permitted on labels bearing the official approval mark. Where a claim is made of pest or disease control for which the product appears to the Advisory Committee to be of doubtful value, the applicant is invited to provide evidence in support of the claim, which otherwise has to be omitted from the label before approval is recommended.

Some products show in their labels references to other named proprietaries; the omission from labels bearing the approval mark of such named proprietaries is required unless or until these have also been approved.

The question of the approval of claims and recommendations applies only to labels attached to containers; the scrutiny of all advertising literature relating to the use of products, if indeed this is desirable, is quite impracticable.

Where an existing label is approved without change, the applicant is able to use on the container of the product a separate label showing the approval mark on condition that the mark becomes incorporated in the main label on the first reprinting. The manufacturer gives an undertaking that following approval no change will be made in the label without the agreement of the Ministry and the Department.

General considerations

When the recommendation of approval and any necessary changes in labels have been made, a Certificate of Approval is granted to the applicant. The Certificate is valid for one year, but may be renewed.

The approval granted relates to the product in the original containers used by the applicant and is operative up to the time of opening the containers. Such a provision clearly is necessary; a product may be transferred from a container in which, for example, it shows satisfactory stability to another which is unsuitable and in which the product deteriorates. With products likely to deteriorate in storage in their original containers, the Advisory Committee needs to be assured of the satisfactory stability of the product over a period covering two seasons of application or, at the most, of two years.

Applicants are charged fees for the approval and the re-registration of products. The fees range from three to five guineas for each product submitted according to the category under which it is considered; the fee for each yearly re-registration is three guineas.

The progress of the scheme

Up to the present time approximately 180 products in 18 groups have been recommended for approval, and of these approximately 150 have appeared in the published approved lists.

So far, products have been considered and classified on the chemical nature of their active constituents. It clearly was impracticable to deal with the many types of insecticides and fungicides at once. In deciding which groups should be dealt with during the early stages of the scheme, attention was given to those classes which were of particular importance for food production under war-time conditions,

for example, the organo-mercury dry seed disinfectants, or to those classes for which specifications were available or could readily be prepared. At the time of the commencement of the scheme, a number of specifications had been published in the Ministry's Bulletins 82 and 122; of these specifications the Joint Panel decided that those for lead arsenate powders, lead arsenate pastes, lime sulphur washes, miscible tar oil winter washes and stock emulsion tar oil winter washes were suitable for the purposes of the scheme. An early start was thus possible by the consideration of these materials, together with the organo-mercury dry seed disinfectants, while the standards required for other groups were under examination.

The approval scheme has provided a valuable stimulus to the preparation of official specifications with accompanying methods of analysis. Apart from their value in providing standards for approval, the specifications and methods are of use from the manufacturers' point of view and in cases of dispute where accepted methods of analysis are required.

Sub-committees of the Joint Panel have from time to time been set up to consider whether the preparation of specifications for particular groups was practicable. Where the reports were favourable, the Joint Panel invited the Ministry and the Association of British Insecticide Manufacturers to set up joint committees to draw up specifications and methods. To date, the following groups have been dealt with in this way: copper sulphate, nicotine, nicotine sulphate, miscible petroleum oil winter washes, stock emulsion petroleum oil winter washes, stock emulsion petroleum oil summer washes for orchard use, stock emulsion petroleum oil summer washes for glasshouse use, Paris Green. The specifications and methods prepared have been accepted by the Ministry, the Association and the Government Chemist, and the groups have been opened for consideration under the scheme. At present, therefore, thirteen groups have been considered on the basis of conformity to official specifications.

Other joint committees of the Ministry and the Association of British Insecticide Manufacturers are at present engaged upon the consideration of specifications and methods for additional groups. In all this work, members of the Advisory Committee and Joint Panel have played notable parts.

In connexion with products in the second category, the Joint Panel has drawn up schedules of the particulars required for the consideration of approval in the following groups: organo-mercury dry seed dressings, Derris and Lonchocarpus insecticides, copper fungicides (exclusive of seed dressings), wetters and spreaders, nicotine dusts, nicotine liquid preparations, derris-petroleum oil washes, thiocyanate-petroleum oil washes, sulphur products, D.D.T. insecticides, D.D.T.-petroleum oil washes, benzene hexachloride insecticides, benzene hexachloride-petroleum oil washes. Applications for the approval of products in these groups have been invited; twenty-six groups of products in all, therefore, have now been opened for consideration. Announcements are made from time to time in the technical chemical, the farming and the gardening press giving the groups in which applications for approval may be submitted.

Two issues of the leaflet giving the full list of products approved have so far been made, and a third issue, bringing the list up to date, will be published shortly. The products approved are listed within the groups alphabetically so as to avoid any suggestion of order of merit. Where desirable, indications are given in the list of the purposes for which the products named are particularly suitable.

An important use of the scheme is in connexion with the Ministry's and the Department's Advisory leaflets and bulletins on plant pests and diseases; where approved products are available in the groups recommended for control, reference to this fact is included in the leaflets and bulletins. Since many thousands of these leaflets are distributed annually, this is likely to do much to bring the scheme to the notice of both the commercial and amateur grower.

Apart from the reference in leaflets and bulletins, the periodic issue of lists in *Agriculture* and the wartime campaign urging the use of organo-mercury seed disinfectants, during which the availability of approved products was pointed out, no attempt has so far been made widely to publicise the scheme. This has been due to two main reasons. In the first place, it was not considered advisable to undertake publicity until all the important groups of insecticides and fungicides had been considered. Secondly, owing to the difficulties of the manufacturers under war conditions in obtaining new containers and labels, the approval mark has not so far appeared to any extent in the retail shop, although it has been used to a greater degree on containers sold to the commercial grower. All the important groups of insecticides and fungicides, however, have now been opened for consideration, and there are signs that the position with regard to the use of the approval mark on containers is changing. It is hoped, therefore, that in the not too distant future, publicity for the scheme will be possible. Not until then can its full value be assessed; the approval scheme will be assured of success when the amateur and commercial grower alike demand products bearing the official approval mark. The scheme, when it was introduced, was regarded rather as experimental in nature, but there now seems to be no reason why the scheme, which provides guidance to the purchaser of insecticides and fungicides without legislation or the testing of products, should not become fully established.

A manufacturer's comments on the approval scheme

By J. R. Booer

May I begin by calling attention to the title of my remarks: 'A manufacturer's comments on the Approval Scheme.' My observations are entirely my own, and I am not the official spokesman of industry. Neither am I expressing the views of any manufacturers' association, nor the considered opinions of any one firm. My only qualifications are: an intimate contact with the Approval Scheme from its pre-emergence stages, and over 25 years' industrial experience.

It may be helpful in the first instance, to consider the position of the manufacturer of insecticides and fungicides in the complete industrial operation of converting primary raw materials into finished products, and delivering them to the user. The first stage consists of the manufacture of the primary raw materials which include various metallic derivatives, oils and distillates, sulphur, fillers and adjuvants, and the grinding of various vegetable products. In some cases these primary materials have to undergo treatment to produce active substances, and in both cases there is usually a further manufacturing stage in which the finished or compounded product, ready for use by the grower, is produced. It is at this stage that the Approval Scheme applies. The manufacturing stages are followed by distribution, usually through wholesale and retail distributors to the grower, or alternatively through contractors of one kind or another. These various activities are not always clearly defined, and several stages in the programme may actually be carried out by one firm. An important fact is that in the great majority of cases, the manufacturer does not deliver the product to the grower, making it necessary for me to mention that I am speaking purely as a manufacturer and not as a distributor.

It is also important to recognize that insecticides and fungicides are part of the much larger business of manufacturing industrial chemicals, and whilst the manufacture of insecticides and fungicides is of absorbing interest to those engaged in it, the weight of chemicals consumed is often quite a small proportion of the production for other industrial purposes. This association, however, carries with it all the creative instincts and stimuli for improvement which have played so great a part in the development and maintenance of the British chemical industry. These influences have made a very definite mark on the chemical control of plant pests and diseases, and an examination of the facts shows that many of the methods now in use were actually introduced by Industry. For example, tar-oil winter washes, organo-mercury seed disinfectants, the modern copper and sulphur fungicides are all of industrial origin. Factory-made lime sulphur relieved the grower of the hazards of the home-made product. The effective introduction of insecticides containing rotenone and pyrethrum was due to industrial enterprise, and still more recently industry has given the grower D.D.T. and benzene hexachloride.

I have recited these facts to justify my next statement, which is that the Approval Scheme is not a dominant factor in the mind of the manufacturer. It has advantages and disadvantages which I shall endeavour to enumerate later, but it must be admitted that the insecticide and fungicide industry made great strides, both qualitatively and quantitatively, before the introduction of the Approval Scheme, and will doubtless go on doing so in spite of it or, as I prefer to hope, in collaboration with it.

Among the general observations that I should like to make is one which I regard as quite uncontroversial and indisputable. I should like to express my unbounded admiration of the courage of all those who sponsored and supported the scheme at a time of very great difficulty, when it would have been so easy to consign it to the uncertain limbo of post-war development.

As a whole, the scheme compares somewhat unfavourably on paper with those in operation in other countries, but as is so often the case with British institutions, the weakness on paper tends to add both to the flexibility and workability of the scheme. Far be it from me to criticize existing legislation, especially if it be under review, but I cannot help feeling that if the pattern of the Fertilizer and Feeding Stuffs Act had been followed, a great deal of effort would have been expended in ensuring that certain minimum standards were maintained, with the possible sequel of increasing the number of products just up to the minimum requirements. On the contrary, the scheme in its present form lays no limit on high quality or good performance. The absence of any policing arrangement is, of course, based on the integrity of the manufacturer, and I have no doubt that time will show by the absence of serious complaints that this policy was justified. In any case, the consumer is safeguarded in that he can call on the services of the public analyst at any time, but I venture to forecast that the novelty of this unnecessary procedure will soon wear off.

The fact that the manufacturer is directly represented on the Joint Panel through the Association of British Insecticide Manufacturers, is of course based on previous experience of the workability of such an arrangement. As merely one member of the Joint Panel, may I be permitted to express the view that that body not only functions efficiently, but is so

truly democratic, that I have no anxiety about the amicable settlement of all the problems that lie ahead.

A most unusual, but in my opinion a very wise aspect of the scheme is that it is voluntary, and a manufacturer's comments on this may be of interest. I am not interested in the sale of worthless products, and the fact that the Approval Scheme does nothing to limit th sale of such products causes me no distress. A .iscriminating public will find out that they are worthless quite soon enough. On the other hand, there may be perfectly good products which are not approved. I usually know the facts in such cases, and if I do not, I usually know my competitor well enough to ask him, and he usually knows me well enough to tell me why he has not sought approval.

Before dealing in detail with the advantages and disadvantages of the scheme, may I say that I do not regard it as an aid to sales. The manufacturer's standard equipment for sales development is more efficient than anything the Approval Scheme can offer. Effective advertising, good personal contacts, a reputation for good service of all kinds, and a sound price structure are not likely to be outweighed by the benefits of the Approval Scheme. I have already pointed out that the manufacturer has little or no contact with the grower, who is the ultimate consumer, and the grower is naturally and justifiably conservative. Certainly, under present conditions, he will continue to use a product which he knows and trusts regardless of whether it is approved or not, and is more likely to be convinced of the merits of new products by demonstrations either at the official centres, on his own land, or on his neighbour's crops, than by the presence of the Approval Mark on a label.

As I have already done much criticizing it might be desirable to deal next with the advantages of the scheme, and it certainly has advantages to the manufacturers, as otherwise they would not have supported it so enthusiastically as they have done. The chief of these advantages to my mind is that the Approval Scheme gives official recognition, on a fuller scale than ever before, to an industry which has rendered a great service to growers in particular and the community in general, at a very low expenditure on chemicals. The grower's purchases of insecticides and fungicides average 1% or so of the value of his crops, and the manufacturer's receipts are, owing to distribution costs, considerably less than the grower's expenditure. Thus, the manufacturer carries a great responsibility on a relatively small income, and the hall-mark of respectability is none the less appreciated, even though it is somewhat belated in comparison with other countries. The scheme has also improved the channels of communication between the Ministry authorities, the advisory officers and the manufacturers. Not only are the advisory officers free from the embarrassment of using proprietary names, but the manufacturers can now use their own trade names in writing without misgiving. I also feel that the operation of the Approval Scheme has facilitated communications between the various manufacturers. These improvements in communications must ultimately benefit all concerned.

The scheme has also had the effect of eliminating certain performance claims which might be open to question, and in this manner some unpleasantness in competition may have been removed.

Another aspect where the scheme may benefit the manufacturer is in the protection it offers him against unjust claims for damages. This very unpleasant aspect of business has largely disappeared during the war, though probably not for ever. Such cases, though rare, can cause the manufacturer a great deal of embarrassment and inconvenience, but when they occur in the future, the fact that an approved product is concerned will have a salutary effect on the negotiations.

A further advantage of the scheme is an internal one which might not be apparent. A manufacturing firm is not a soulless entity. It comprises a collection of individuals whose views do not always coincide. The Approval Scheme has provided standards which can be used internally to maintain quality and to safeguard products against the encroachments of the enterprising amateur.

Before turning to the disadvantages of the scheme I would ask that it be noted that the principal advantages are philosophical rather than material, and I believe that this is what the sponsors of the scheme would desire.

In dealing with the disadvantages of the scheme it is admitted that many of them have been magnified by war-time difficulties, but it should not be too easily assumed that all the difficulties will disappear with war-time limitations. Many difficulties have been encountered in connexion with labelling of packages, and as the label is so important an item in the Approval Scheme I propose to deal with this first.

As the grower does not buy direct from the manufacturer, the label is the manufacturer's only means of communication with the grower. The preparation of a label, therefore, requires the utmost care. Not only must it be scientifically accurate; it must convey necessary information to the grower in terms which he can understand. Here is a possible point of divergence between the manufacturer and the advisory committee. Furthermore, the label should be attractive enough to help sell the goods, and in this connexion the maker's trade mark, on which a great deal of his goodwill depends, must be effectively displayed. Thus, the preparation of a label calls for the

combined efforts of the scientist, the publicity expert and the artist, and the overhead costs of producing a new label are not inconsiderable. The Approval Mark must be a photographic reproduction, and this is not always convenient. Although some easement may be expected in the supplies both of paper for labels and in sizes and shapes of containers, I would still ask that the question of revising a label should be treated with respect, and that the question of scrapping existing supplies of labels should be treated with more respect. It is probable that there will be a trend towards printed tins, and here the technical complications are still greater, necessitating still more respect when alterations are contemplated or proposed.

Included in the disadvantages of the scheme is a peculiar commercial effect resulting from the approval of products in groups. I have pointed out the great importance of the distributor in the industrial scheme, and an argument used not infrequently by distributors is based on the assumption that all the approved products in one group are equally good, and he is therefore justified in buying the cheapest. Alternatively, the distributor may try to persuade the manufacturer to bring his price down to that of the cheapest product in the group. This is neither the time nor the place to debate the merits or demerits of price fixing between manufacturers, but it is, nevertheless, appropriate that I should point out this aspect which is a defect in the scheme from a manufacturer's point of view. I would suggest that more general appreciation is needed for the fact that whilst the Approval Mark only means that the product will fulfil the claims made on the label, there is still room for great diversity of performance within a group of products, all of which reach the minimum standard claimed. I think if this point were fully explained to the distributing trade and to the users, it would maintain the flexibility of choice which is the greatest stimulus to the manufacturer to improve his product above the agreed minimum of performance. Those responsible for the scheme will probably say that questions of price are not their concern, and others will contend that the remedy for this trouble is to be found by raising the present standards. This may be a popular line of thought in some circles, but I submit that the question of raising the present standards is not nearly as easy as it appears. Without embarking on this subject too far, I would like to point out that manufacturers are fully entitled to protect by patent any novel improvement in their products, and can in this way make improvements which may not be accessible to other manufacturers in the same group. I would ask that this point should be borne in mind when the question of raising standards is discussed. I would also suggest that possible repercussions resulting from the removal of a product from the approved list should be most carefully considered, as the manufacturer concerned may have other products on the approved list.

A manufacturer's views on how the value of the scheme might be increased may not be out of place. Certainly, every effort should be made to widen the scheme, and to approve as many groups of products as is feasible. I am aware that this is being done, but would take the opportunity of pointing out that the scheme cannot attain its full value until there is an approved product available against every insect pest and fungus disease with which the grower has to contend, and which can be controlled by chemical means.

It will be admitted that the public value of the scheme is directly proportional to the number of interested persons who know about it. This raises the question of publicity, although whether the time has yet arrived to give full publicity to the scheme may be a controversial point. My personal opinion is that it would not be advisable to wait until there is complete coverage, and I would suggest that a dividing line should be drawn in the very near future. A concerted attempt should then be made to impress all those concerned with the value of the scheme to them. The manufacturers are already sufficiently familiar with this, and the necessity for bringing the matter to the notice of the grower is appreciated. I would, however, add to this, that it is of the utmost importance to convert all sections of the distributing community, who, I believe, are as yet not wholly convinced about the scheme. A great deal of good could be done if the distributors could be persuaded to advise the grower to use approved products wherever possible. The collaboration of the manufacturer in any publicity scheme would be available if required, and it is worth mentioning that the manufacturers are very experienced in publicity as the result of having to spend their own money on it. In this connexion, a source of reliable information is available through the manufacturers' association.

I trust that the various criticisms that I have made will not be regarded as deprecating the scheme. I am, and always have been, an enthusiastic supporter of it, and it is for this reason that I have submitted my opinions on its limitations and its defects. As I am expressing personal views it would be inappropriate for me to suggest remedies which might not be acceptable to other manufacturers. The difficulties can be overcome by discussion, and I have no hesitation in assuring this meeting that when such discussions take place, the necessary goodwill will be available from the manufacturing community, myself included.

The approval scheme as seen by a specialist advisory officer

By W. A. R. Dillon Weston, *School of Agriculture, Cambridge*

I believe that this welcome innovation of a voluntary approval scheme is one of the most significant advances yet made for the benefit of agricultural and horticultural practice. It is clear (or at least it would seem clear) that this scheme enables county or provincial advisers to recommend, without any stigma of bias or favouritism, certain well-known products which are essential for the control of plant pests and diseases. At least that is the theory of it, but does it work in practice? Admittedly, it withholds approval from products of very dubious value, but what reputable firm would sell these? The credulous instinct of mankind will not be changed in a day, and quack remedies will continue to be sold until such time as they are ridiculed or legally suppressed. It is necessary I think to ignore such products when viewing this voluntary approval scheme, and to confine any discussion to relatively reliable materials made by firms with a reputation at stake. As I am interested in seed disinfectants it will be more appropriate if I consider the matter in relationship to the organo-mercury seed dressings. When Group F was first initiated two or three products only were approved, but, as might be expected, more have been added to it, and more will probably appear in the future. If I remember rightly there are now eight approved organo-mercury dressings, a wide range therefore of *different* products from which the farmer can choose. First, however, are they all different? May it not be that some are fungicidally of similar composition, but under different brand names? If this is so, or if this development took place, I think that confusion must arise. There is now no mystery or magic about the composition of these dressings, and I suggest that it may be in the best interests of all concerned if the specification of an approved product is disclosed on its container, the fungicidal constituent(s) being stated and the percentage(s). The question as to whether or not certain brands were similar or dissimilar would not then arise.

It may be argued that the greater the number of products approved by the committee the more they will become distributed, and, as a consequence, the standard of disease control throughout the country will be improved. I incline to the opposite view. If these products are not of a uniformly high standard then the more there are, the more likely it is that disease control will be lower and not higher. Further, the manufacturers of the better approved dressings may be penalized in that they are forced to compete with a wide range of approved but relatively less effective dressings.

How effective are these seed dressings in preventing the diseases which they are stated to control? Are they all equally good, or do they vary? If they vary, is the variation within wide or narrow limits? My colleague, Mr A. R. Loveless, has prepared figures showing some of the results from our test trials at Cambridge (Table 1). These trials were designed to assess the relative efficiency of the various proprietary organo-mercury seed dressings in the control of seed-borne diseases of cereals by comparing their performance *in the field*. The trials consisted of blocks composed of rod row plots, some of which were allocated to controls and the remainder to the treatments—one row per treatment. The plots were randomized within each block and the blocks replicated eight times. The grain was dressed at the rates recommended by the manufacturers, and an equal volume was sown per row. In the Table the officially approved products in Group F are 'in code' and are designated by the numerals 1 to 7; the disease estimates are the mean values of the eight replicates.

You will agree, I think, that some seed dressings appear to be consistently better than others. Now if you were a farmer you would wish to use the best product available. But what is to be your guide—an approved product? There are many of them, there may be many more! I do not wish to lead you needlessly into complexities or to debate the ethics of nice professional conduct—but does an adviser knowing the relative, and sometimes changing, values of these dressings advise the use of any organo-mercury dressing, or does he suggest to his client one of the more efficient, and name the brand? The adviser is a specialist consultant, and when farmers approach him for advice they require the best and latest information. It is the best and better farmers who seek advice and not the worst, and it is no helpful guide to them to suggest that they use one or other of these approved products. They wish to know more than that, and rightly so I think. It is to be hoped, of course, that research and active competition will lead to improvements in these dressings and that eventually the general standard of performance will be higher. But in the meantime what guide is there for the farmer, and what is to be the incentive to provoke this healthy competition and research? Might it not be a wise plan to approve these—and other seed dressings—only after published results of field tests have demonstrated that they give an adequate control of seed-borne diseases? The crux of this matter is now reached and it is clear that many debatable and interesting points of principle

TABLE 1. *Seed disinfection trials on the Cambridge University Farm*, 1941–5

Product ...	1	2	3	4	5	6	7	Control
				Mean percentage infection of bunt				
1941	2·12	0·05	0·06	0·00	1·08	2·43	—	36·12
1942	0·05	0·00	0·00	0·00	0·04	0·00	—	2·62
1943	2·31	0·26	0·75	0·81	0·73	5·51	—	50·37
1944 (*a*)	1·82	0·19	1·37	0·13	0·18	0·29	—	80·38
(*b*)	—	0·35	—	—	—	—	0·25	14·97
1945	0·59	0·10	0·95	0·16	0·16	0·13	0·29	18·87
				Mean percentage infection of leaf spot				
1941	—	—	—	—	—	—	—	—
1942	10·30	4·26	6·78	0·37	7·25	0·91	—	23·94
1943	0·54	0·00	0·32	0·82	0·10	2·02	—	5·91
1944	9·15	0·36	9·18	0·00	8·73	0·00	6·33	13·72
1945	2·25	0·00	0·22	0·00	0·23	0·00	1·89	17·90
				Mean percentage infection of oat smut				
1941	—	—	—	—	—	—	—	—
1942	—	—	—	—	—	—	—	—
1943 (*a*)	1·33	0·09	0·23	0·50	0·15	4·03	—	4·54
(*b*)	4·44	3·07	5·69	6·31	3·36	6·11	—	12·15
1944	—	—	—	—	—	—	—	—
1945	1·01	0·20	0·41	0·21	0·09	0·70	0·88	2·44

N.B. In the years in which no results are tabulated either the trial was omitted or the control figure was so low that the figures for the treatments were of no significance.

and detail, or strategy and tactics, arise. Some of these can now be considered. A central experimental testing station, official or unofficial, would seem to be necessary. How would this be designed and how would it operate? I visualize it more in the nature of a joint experimental station to which manufacturers (or private research workers) would submit likely products for test. We must remember, I think, that research on seed disinfection is continuous, it did not end with the introduction of the organo-mercurials. The mere testing of a product for approval would be but one phase of the work, for besides this there would be research and detailed enquiry into many fundamental problems of this subject. Would a station of this kind be any let or hindrance to the smooth working of commercial research? Would the technicians and advisers of these firms resent such an innovation? It is perhaps pertinent to say here that a 'voluntary unofficial station' for the testing of seed disinfectants (the composition being previously declared) has been operated at Cambridge for many years, the first organo-mercurial being treated as long ago as 1924. It may be argued that a station such as I have indicated is unnecessary and that these dressings could be tested, on a co-operative basis, by provincial advisers. I suggest that this method of approach is undesirable. Was it not one of the main preoccupations of advisers some 10 years or so ago—the determination of the relative merits of product *A* versus product *B*—the composition of both being unknown? Let us hope that so unscientific a procedure will be resisted in the future.

But what standards are to be adopted, if it is assumed that some official or unofficial testing scheme is desirable? What constitutes an adequate control? These are questions more suitable for debate in committee than for discussion here. It will suffice to say that a scale of standards could be devised for each specific disease and these regulated according to the degree of infection arising from untreated samples. There are no great technical difficulties here. The standard adopted, however, must be high.

Lastly, and as I have already stated, I consider the voluntary approval scheme a most meritorious conception, but I am afraid that it may defeat its own purpose by the sanctioning of a plethora of good, but not sufficiently good, products. May it not be that once a product is approved complacency may weaken incentive? It is true that perfection is never attainable, but at the same time there is nothing quite so good as the best, and that is the view which I take as a specialist adviser.

The grower's impressions of the approval scheme

By O. G. Dorey, *Essex Institute of Agriculture, Chelmsford, Essex*

During the last week or two, I have mentioned to a number of fruit-growers the approval scheme for proprietary products for the control of plant pests and diseases—and their reactions have varied from the otherwise enlightened grower who thought it would be a good idea if something of the kind was brought into force, to the growers at the other end of the scale who showed a willingness to enter into quite a heated argument mainly in this case on what they consider to be the demerits of the scheme.

I found, therefore, a section of growers who had virtually no knowledge of the scheme—and another section who were fully aware of it and found it wanting. The fact that there are growers who know nothing about this scheme, is capable of a number of interpretations, not the least of these being that as there are high quality products available which satisfy the claims made by the manufacturers, these growers have not felt the need of a scheme. Nevertheless, it is significant that a number of leading growers in the county know nothing of this scheme—so lack of publicity may mean that growers are not getting the benefits of a scheme which is designed in part to safeguard their interests.

It is a fact then that many growers do not know that the scheme exists—perhaps the label is not sufficiently striking to publicise its approval—or is it that the label is on occasions missing from the package?

It is vitally necessary, however, that the publicity the scheme receives should be of its good points—and not of its *deficiencies*.

If one of these growers were to read the official article giving details of the scheme, he would find that the declared objects of this were:

(i) that persons professionally concerned with giving advice on the control of plant pests and diseases should be able, on reasonably sure foundations, to recommend by name an appropriate range of products;

(ii) that the consumer should be able to see at a glance that the product he is purchasing is a good one for his purpose.

Now both these objects are mostly highly desirable and if they can be implemented, the scheme must have a value of which these growers could not afford to be ignorant. There is little doubt that the publication of lists of approved insecticides and fungicides is a help to the adviser and also to the grower who is perhaps inexperienced. It has always been a handicap when giving advice to growers that proprietary brands could not be mentioned. Lists can now be kept of approved products and handed or sent to the grower. It is so much more satisfactory to be able to say to the grower who is new to the job you should use this wash at such and such concentration, and these are the firms who supply the right material. One has so often recommended a certain wash to a grower, only to find that he finishes up by buying something quite different—if you can supply lists of the washes with the trade names, then there is a greater probability that the grower will get the right wash for the right job.

Both grower and adviser these days are faced with a whole host of proprietary products; each firm may issue some straightforward chemical—under their own trade name. There are, no doubt, sound commercial reasons for so doing and to try and advertise dichloro-diphenyl-trichoroethane as such would, no doubt, be bad advertising and expensive in space—I must confess, however, that I find it a little irritating to have to guess what some of these proprietaries really are and tend to distrust them until I know.

Now some of these growers who were unaware that a scheme was already in existence made some comments on what such a scheme should do. The first was that the vendors of proprietary products should be compelled to disclose the complete analysis of such products—I give that in the words of the grower—well, it may be of course that the complete analysis of the spray material would be meaningless to the grower—but what he obviously wants is declaration of the composition of the material, if possible in terms of amount of the active ingredient so that he can know what it will do and compare it with others. This may be easy with some materials but perhaps not by any means with all. One grower suggested to me that a system of evaluating insecticides and fungicides similar to the unit value method of determining fertilizers should be evolved. There is no reason why the same principle should not be used in the case of some materials where the value of the product as an insecticide or fungicide is based only on the amount of a simple ingredient being present and not on any other factors. Another comment was that proprietary products should be put on a period of trial, before being approved. The thorough testing out of materials which are unknown is sound provided that there is no unreasonable delay in getting materials on to the market and available to growers.

The present scheme allows for approval of a product which conforms to an agreed specification or one in which the chief ingredients are declared—this should go far to satisfy the growers' demands. What then are the reasons for the indignation on the part of the section of growers who say the scheme is wanting? The criticisms of these growers are that in certain fundamentals, the scheme is lacking and that thereby it fails to safeguard the growers' interests. The two main criticisms are:

(i) The approval of the Ministry is given only on a declared specification submitted by the manufacturers and not substantiated by independent or official analysis; or, as another grower put it, anyone can get approval without the material being subject to official test.

(ii) No independent or official check is maintained on the standard of the products.

Now it is clear that the existing voluntary scheme does not provide for either of the above conditions, but it is felt that the scheme would be improved if they were adopted. What happens if a fruit-grower purchases a spray material approved under the scheme and, following application of the wash to his trees, they are severely damaged—all the normal precautions and conditions having been observed and the only cause under suspicion being the composition of the wash? If he wishes to test this he will find that he must consult the public analyst and *he* will probably only deal with an *unopened* container. The weakness of the grower's position here is obvious. He next finds that the scheme makes no provision for legal action being taken against the manufacturer—the grower has imagined that it would be an offence for the materials not to be up to specification. No official action can be taken apart from withdrawing approval of the products and then only those found not to conform to the specification after analysis by the public analyst. Being thoroughly inquisitive he delves further into the provisions of the scheme, is rather surprised to find that no precautions at all are taken to see that the product approved is tested officially at the outset or that a check is taken from time to time afterwards to see that the specification has been maintained.

The care and attention given by makers to produce spray materials which are thoroughly reliable and conforming to agreed standards is known and appreciated—as one grower, who had never heard of the scheme, put it to me—'hitherto I have relied on the integrity of the manufacturers and it is to their credit that I have as yet had no cause to complain'. But it is, nevertheless, felt that an official scheme should carry with it a greater official obligation than exists at present to safeguard the interests of the grower. These growers do consider that if the scheme is to be of real value, in addition to its admitted value to grower and adviser when choosing and recommending a spray material, official samples should be taken from time to time to ensure that the specification was being adhered to. I have no doubt that, even if such were admitted to be desirable, it would be found to be possible only under some completely reorganized scheme of much wider scope—involving perhaps even the necessity for a compulsory scheme with a declaration of analysis, and sample checks being taken, etc. An elaborate scheme such as this may even not be possible for technical reasons.

No scheme whether simple or elaborate, whether voluntary or compulsory, can prevent occasional mishaps, but these growers do feel that the present scheme perhaps has more advantages for the manufacturer than the grower.

Report of the Council of the Association of Applied Biologists for the year 1945

The Officers and Council of the Association were as follows:

President: C. B. Williams, M.A., Sc.D.

Vice-Presidents: Prof. W. Brown, D.Sc., F.R.S., W. C. Moore, M.A.

Hon. Treasurer: W. C. Moore, M.A.

Hon. Secretary: W. P. K. Findlay, D.Sc.

Hon. Asst. Secretary: H. Shaw, M.Sc., Ph.D.

Hon. Editor of the Annals of Applied Biology: Prof. W. B. Brierley, D.Sc.

Hon. Asst. Editor: R. W. Marsh, M.A.

Council: L. A. Allen, Ph.D., G. H. Bates, D.Sc., F. C. Bawden, M.A., J. R. Busvine, Ph.D., M. Cohen, Ph.D., G. V. B. Herford, B.A., J. T. Martin, D.Sc., E. W. Mason, M.A., H. W. Miles, D.Sc., M. H. Moore, M.Sc., R. T. Pearl, B.Sc., Miss A. P. Wilson, A.R.C.S.

Once again the Council is able to report another year of successful progress for the Association. The membership has continued to increase, 31 new ordinary members having been elected during the year. The end of the war finds the Association in a stronger position than it has ever been before: a great contrast to the position at the end of the 1914–18 war, when the total membership was only 130 and the Association was hard pressed for funds to carry on the publication of the *Annals*. The total membership, including 11 honorary members, now stands at 395, an increase of 23 over the previous total and 83 more than in 1941. It is with regret that the Association has to record the deaths of K. Fisher, who had been a member since 1919, of G. Howard Jones who lost his life in an air crash, and of our distinguished honorary members, L. R. Jones of Wisconsin, U.S.A. and N. I. Vavilov of Leningrad. Two new honorary members—Prof. E. Gram (Denmark) and Dr Quanjer (Holland) have been elected.

Five well attended meetings were held in London, an average of 48 members and 46 visitors recording their names as present at each meeting.

On 29 June, 41 members visited Rothamsted Experimental Station at the kind invitation of the Director and spent an interesting day examining exhibits in the laboratories and glasshouses.

The following papers were presented to the Association during the year:

23 Feb. Presidential address, by Prof. W. Brown: *Plant pathology—teaching and research.*

23 Mar. Joint meeting with the Genetical Society. *Symposium on genetical relations of plants and animals to their pests and diseases.* C. D. Darlington: General introduction. J. Hammond: Constitution in cattle in relation to disease. M. S. Pease: Observations on heritable disease in poultry. J. G. Carr: Heritable susceptibility of poultry to cancer virus. W. Black: Inheritance of blight resistance in potatoes. G. Cockerham: Genetical aspects of resistance to potato viruses. T. J. Jenkin: Disease and pest problems at the Welsh Plant Breeding Station.

5 Oct. *Symposium on some agricultural uses of D.D.T.* C. T. Gimingham: General introduction. G. H. L. Dicker: Apple blossom weevil and its control by D.D.T. M. Cohen: Experiments with D.D.T. smokes. K. G. Coghill and W. Steer: The control of flies in farm buildings by D.D.T. J. B. Cragg: The control of sheep blowfly by D.D.T. dips. G. B. S. Heath and G. G. Mitchell: Experiments on the control of sheep ticks by D.D.T.

9 Nov. *Discussion on factors controlling flowering.* Prof. F. G. Gregory: The need for research into the factors controlling flowering. Prof. G. McLean Thompson: The events of flowering as viewed by a morphologist. Prof. R. H. Stoughton: Photoperiodic induction in *Tithonia speciosa*. Miss O. N. Purvis: Vernalization. O. V. S. Heath: Some external and correlative factors controlling flowering in the onion plant.

7 Dec. *War-time problems of seed supply in Great Britain.* H. Hunter: General introduction. L. E. Cook: Survey of Britain's seed supply in war-time. W. C. Moore: Seed-borne diseases.

The Council wishes to take this opportunity of expressing its thanks to the authorities of the Imperial College of Science and Technology and of the London School of Hygiene and Tropical Medicine for so kindly affording the Association accommodation for its meetings.

The Association has been represented at preliminary meetings called by the Biochemical Society to consider the formation of a Council to assist in co-ordinating the activities of various biological societies, and a Biological Council on which the Association is represented came into being on 21 November 1945. While the immediate objects of this Council will probably be limited to such matters as the

circulation of information about the dates and subjects of meetings of the societies, it is envisaged that the scope of the Council's work may enlarge and that editorial problems relating to the publications of the various societies may fruitfully be discussed in common.

Plant Pests and Diseases Committee: Report for 1945

The rules governing the activities of the Committee provide that four members shall retire annually; since in 1944 the Committee had not been in existence for a full year, the Council decided that the constitution of the Committee as given in the *Annals*, 1945, p. 190, should remain unchanged for 1945.

Four meetings have been held during the year. At the first J. T. Martin and W. G. Keyworth were re-elected Chairman and Secretary respectively.

The study of the methods of recruitment and training of plant pathologists in Great Britain has been concluded. A report on the subject with modifications has been adopted by Council, sent to the Loveday Committee and other official and academic bodies, and will shortly be published in the *Annals*.

The Sub-Committee formed in 1944 in collaboration with the Ministry of Agriculture Conference of Advisory Entomologists has made valuable progress in the compilation of a list of common names of British insect and other pests. The first section of the list, dealing with slugs and snails, eelworms, Coleoptera, Diptera and Hymenoptera has been completed and will shortly be submitted for publication.

Hon. Editors' Report for 1945

Vol. **32** of the *Annals of Applied Biology* comprised pp. viii + 380 and 12 Plates as against pp. viii + 387 and 11 Plates for vol. **31**. Including papers and abstracts published in *Proceedings*, vol. **32** contained 65 communications, 50 being by Members of the Association. The subjects may be roughly classified as follows: general applied botany, 7; mycology and fungus diseases, 11; bacteriology and bacterial diseases, 3; viruses and virus diseases, 4; general zoology, 7; entomology and insect pests, 12; helminthology and nematode diseases, 5; applied microbiology, 3; plant protection, insecticides and fungicides, 5; general, technique, etc., 8.

Publication of the *Annals* was delayed owing to production difficulties and the several numbers of vol. **32** were issued on the following dates; No. 1, 7 May 1945; No. 2, 30 July 1945; No. 3, 21 Nov. 1945; No. 4, 16 Jan. 1946.

In consequence of the increase in the number of manuscripts submitted during the year, Council has decided that preference in publication must in future be given to members' papers.

With the publication of No. 4, Prof. W. B. Brierley retired from the Hon. Editorship of the *Annals* which he has held for 25 years.

Report of the Honorary Treasurer for the year ended 31 December 1945

During the year ended 31 Dec. 1945, the amount received for subscriptions and entrance fees, including arrears, was £461. 18*s*. 6*d*., which was £20. 11*s*. 6*d*. more than in 1944. There were only six subscriptions remaining unpaid at the end of the year.

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £875. 2*s*. 6*d*., an increase of £150. 5*s*. 0*d*. This considerable increase was due primarily to the demand for the extra copies available as a result of raising the printing order late in 1944 from 700 to 800 copies. The volume contained 380 pages compared with 387 pages in 1944 and the cost of producing it was £1373. 4*s*. 7*d*., or only about £27 more than in the previous year. There was an unprecedented demand for back volumes and parts, which realized £312. 10*s*. 4*d*., compared with £77 in 1944: this greatly affected the balance against the *Annals* on the year's working, the amount being £173. 17*s*. 9*d*. compared with over £500 the previous year.

Income for the year exceeded expenditure by £248. 4*s*. 2*d*. and in addition the Association received a grant of £175 through the Royal Society from the special Rockefeller Foundation Grant in aid of Scientific Publications. This was the fifth annual grant from this source and Council acknowledges its receipt with gratitude. After all obligations had been met, the assets of the Association at the end of the year amounted to £1518. 15*s*. 6*d*., of which £755. 14*s*. 4*d*. was represented by National Savings Certificates and £97. 10*s*. 0*d*. by the estimated value of the stock of the *Annals*. On 31 December there was also a sum of £671. 18*s*. 1*d*. to the credit of the Association in a Post Office Savings Bank Account. In view of the partial lifting of the restrictions on paper the Council has already sanctioned considerable additional expenditure on the *Annals* in 1946 in an endeavour to reduce a temporary delay in publication.

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1945 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.
To Estimated Value of Stock, 1 January 1945	116	3	0	By Sales—Current Volume	875	2	6
To Cambridge University Press	1373	4	7	By Sales—Back Volumes, Parts and Sets	312	10	4
				By Sales of Reprints and Adverts	30	7	0
				By Estimated Value of Stock, 31 December 1945	97	10	0
				By Balance, carried down	173	17	9
	£1489	7	7		£1489	7	7

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1945 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.	£	s.	d.
To *Annals of Applied Biology*, balance brought down	173	17	9	By Members' Subscriptions:						
To Printing and Stationery	11	2	3	Arrears	3	15	0			
To Postages and Cheque Stamps	17	4	11	Entrance Fees	19	8	6			
To Subscription—Parliamentary Science Committee	10	10	0	Current	438	15	0			
To Sundry Out-of-Pocket Expenses of Editors, Secretaries and Treasurer	19	19	0					461	18	6
To Expenses of Plant Pests and Diseases Committee	9	2	5	By Interest Receivable:						
To Audit Fee Reserve	5	5	0	National Savings Certificates	20	0	6			
To Balance, being Excess of Income over Expenditure for the Year	248	4	2	Post Office Savings Bank Account	13	6	6			
								33	7	0
	£495	5	6					£495	5	6

BALANCE SHEET, 31 DECEMBER 1945

Liabilities and Surplus	£	s.	d.	£	s.	d.	Assets	£	s.	d.	£	s.	d.
Sundry Creditors:							Cash at Bank:						
Cambridge University Press	180	4	9				Current Account	272	1	0			
Audit Fee Reserve	5	5	0				Post Office Savings Bank Account	671	18	1			
Sundry Expenses	25	13	8								943	19	1
				211	3	5	500 National Savings Certificates				755	14	4
Subscriptions and Entrance Fees, paid in advance				42	4	6	Stock of *Annals of Applied Biology* at estimated value				97	10	0
Life Membership Fund Reserve				25	0	0							
Excess of Assets over Liabilities:													
As per Balance Sheet of 31 December 1944	1095	11	4										
Add: Rockefeller Foundation Grant (per Royal Society)	175	0	0										
Balance of Income and Expenditure Account for 1945	248	4	2										
				1518	15	6							
				£1797	3	5					£1797	3	5

W. C. MOORE, *Hon. Treasurer*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed) H. J. COX & CO. *Incorporated Accountants* } *Auditors*

HARPENDEN, 16 *February* 1946